# Canine Cardiac Troponin I Significantly Complements Established Prognostic Composite Score In Dogs With Systemic Inflammation.

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*Published in:*
2012 ACVIM Forum Research Abstracts Program

*Publication date:*
2012

*Document version*
Early version, also known as pre-print

*J Vet Intern Med* 2012;26:690–822

# 2012 ACVIM Forum Research Abstracts Program

New Orleans, Louisiana, May 30 – June 2, 2012
Index of Abstracts

## ORAL PRESENTATIONS – Thursday, May 31

| Time | # | Presenting Author | Abstract Title |
|---|---|---|---|
| **SMALL ANIMAL – CARDIOLOGY**** | | | |
| 9:00 am | C-1 | Paul Mõtsküla | PROGNOSTIC VALUE OF 24-HOUR AMBULATORY ECG (HOLTER) MONITORING IN BOXER DOGS |
| **9:15 am** | **C-2** | **Ryan Fries** | **COMPARISON OF TRANSTHORACIC TWO-DIMENSIONAL ECHOCARDIOGRAPHY, THREE-DIMENSIONAL ECHOCARDIOGRAPHY, TRANSESOPHAGEAL ECHOCARDIOGRAPHY, COMPUTED TOMOGRAPHY ANGIOGRAPHY, AND GATED MAGNETIC RESONANCE IMAGING IN NORMAL CANINE HEARTS** |
| **9:30 am** | **C-3** | **Bryan Bottorff** | **USE OF COMPUTED TOMOGRAPHY IN THE ASSESSMENT OF DOGS WITH PERICARDIAL EFFUSION** |
| **9:45 am** | **C-4** | **Kristen Parker** | **DIRECT ARTERIAL BLOOD PRESSURE MEASUREMENT USING A CAROTID CATHETER IN COMPARISON TO OSCILLOMETRIC AND DOPPLER METHOD** |
| *BREAK* | | | |
| 10:30 am | C-5 | Sara Johns | CAN STRAIN RATE IMAGING DIFFERENTIATE PHYSIOLOGIC FROM PATHOLOGIC HYPERTROPHY IN THE DOG? |
| **10:45 am** | **C-6** | **Carley Saelinger** | **OPTIMIZATION OF IMPLANTABLE CARDIOVERTER DEFIBRILLATOR (ICD) DEFIBRILLATION THRESHOLDS IN DOGS** |
| **11:00 am** | **C-7** | **SeungWoo Jung** | **ATRIAL FIBRILLATION IS A NEGATIVE PROGNOSTIC INDICATOR IN LARGE BREED DOGS WITH MYXOMATOUS MITRAL VALVE DEGENERATION AND CONGESTIVE HEART FAILURE** |
| 11:15 am | C-8 | Hannah Stephenson | MALIGNANT VENTRICULAR ARRHYTHMIAS ARE ASSOCIATED WITH DILATED CARDIOMYOPATHY IN GREAT DANES |
| 11:30 am | C-9 | Ioannis Giatis | UTILIZATION OF TRANSESOPHAGEAL ATRIAL PACING TO OBTAIN SINUS NODE RECOVERY TIME IN DOGS |
| 11:45 am | C-10 | Ashley Saunders | LONG-TERM EVALUATION OF CARDIAC SIZE AND FUNCTION IN 71 DOGS FOLLOWING PATENT DUCTUS ARTERIOSUS CLOSURE |
| 12:00 pm | C-11 | Rebecca Langhorn | CANINE CARDIAC TROPONIN I SIGNIFICANTLY COMPLEMENTS ESTABLISHED PROGNOSTIC COMPOSITE SCORE IN DOGS WITH SYSTEMIC INFLAMMATION |
| **12:15 pm** | **C-12** | **Keith Blass** | **CLINICAL EVALUATION OF THE 3MTM LITTMANN® MODEL 3200 ELECTRONIC STETHOSCOPE IN CATS** |

**Also see Cardiology abstracts C-13 – C-26 (Thursday, May 31, 2:15 pm – 6:15 pm)* Boldface type indicates ACVIM Resident Research Award eligibility.

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**SMALL ANIMAL - ENDOCRINOLOGY**

| | | | |
|---|---|---|---|
| 9:00 am | EN-1 | Tim Williams | RESTORATION OF EUTHYROIDISM IN MEDICALLY TREATED HYPERTHYROID CATS WITH IATROGENIC HYPOTHYROIDISM (IH) IMPROVES RENAL FUNCTION |
| 9:15 am | EN-2 | Mark Peterson | THYROID SCINTIGRAPHY FINDINGS IN 917 CATS WITH HYPERTHYROIDISM |
| **9:30 am** | **EN-3** | **Bethany Sabatino** | **AMINO ACID, IODINE, SELENIUM, AND COAT COLOR STATUS AMONG HYPERTHYROID, SIAMESE, AND AGE-MATCHED CONTROL CATS** |
| 9:45 am | EN-4 | Aline Vieira | EFFECT OF THE STAGE OF CHRONIC KIDNEY DISEASE ON SERUM TOTAL THYROXINE IN CATS |
| *BREAK* | | | |
| **10:30 am** | **EN-5** | **Eric Walsh** | **ASPART INSULIN CONSTANT RATE INFUSION FOR TREATMENT OF DOGS WITH DIABETIC KETOACIDOSIS** |
| 10:45 am | EN-6 | Mia Reeve-Johnson | DETERMINATION OF REFERENCE VALUES FOR CASUAL BLOOD GLUCOSE CONCENTRATION IN CLINICALLY-HEALTHY, AGED CATS MEASURED WITH A PORTABLE GLUCOSE METER FROM AN EAR OR PAW SAMPLE |
| 11:00 am | EN-7 | Erin Mooney | EARLY-ONSET HYPOADRENOCORTICISM IN A KINDRED OF POMERANIANS |
| **11:15 am** | **EN-8** | **Rebecca Haviland** | **CLINICAL FEATURES OF HYPOADRENOCORTICISM IN SOFT-COATED WHEATEN TERRIERS: 72 CASES (1979–2011)** |
| **11:30 am** | **EN-9** | **Emily Skovira** | **THE EFFECT OF SURGERY ON THE PITUITARY-ADRENAL AXIS IN DOGS** |
| 11:45 am | EN-10 | Pascale Smets | LONG-TERM FOLLOW-UP OF RENAL FUNCTION IN DOGS WITH ACTH-DEPENDENT HYPERADRENOCORTICISM (ESVE Award Winner) |
| **12:00 pm** | **EN-11** | **Jessica Timian** | **EVALUATION OF A LONG-ACTING SOMATOSTATIN RECEPTOR LIGAND FOR THE TREATMENT OF FELINE ACROMEGALY** |
| 12:15 pm | EN-12 | Linda Martin | BASAL ALDOSTERONE CONCENTRATIONS AND RESPONSE TO ACTH STIMULATION IN HEALTHY AND CRITICALLY ILL DOGS |

**SMALL ANIMAL – NEPHROLOGY / UROLOGY**

| | | | |
|---|---|---|---|
| 9:00 am | NU-1 | Meryl Littman | PROTEIN-LOSING NEPHROPATHY ASSOCIATED WITH MUTATIONS IN NPHS1 (NEPHRIN) AND KIRREL2 (NEPH3/FILTRIN) IN SOFTCOATED WHEATEN TERRIERS |
| **9:15 am** | **NU-2** | **Cara Horowitz** | **PRE-OPERATIVE PREDICTORS OF OUTCOME FOR CATS WITH SUCCESSFUL MANAGEMENT OF URETERAL OBSTRUCTIONS VIA URETERAL STENTING OR A SUBCUTANEOUS URETERAL BYPASS DEVICE** |
| **9:30 am** | **NU-3** | **Sarah Stewart** | **EVALUATION OF WHITE COAT HYPERTENSION IN DOGS AND CATS USING THE petMAP OSCILLOMETRIC BLOOD PRESSURE MONITOR** |
| **9:45 am** | **NU-4** | **Danielle Weinstein** | **THE EFFECT OF BODY POSITION ON INDIRECT SYSTOLIC BLOOD PRESSURE MEASUREMENT IN DOGS** |
| *BREAK* | | | |
| 10:30 am | NU-5 | Rebecca Geddes | FEEDING A RENAL DIET IS ASSOCIATED WITH A REDUCTION IN PLASMA FIBROBLAST GROWTH FACTOR 23 (FGF-23) IN CATS WITH STABLE CHRONIC KIDNEY DISEASE (CKD) |
| 10:45 am | NU-6 | Adam Eatroff | ACUTE KIDNEY INJURY IN DOGS HOSPITALIZED IN THE INTENSIVE CARE UNIT: A PROSPECTIVE STUDY (INTERIM ANALYSIS) |
| 11:00 am | NU-7 | Thierry Francey | LONG-TERM RENAL OUTCOME OF DOGS WITH ACUTE KIDNEY INJURY |

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|---|---|---|---|
| **11:15 am** | **NU-8** | **Amy Lomas** | **ACUTE AND CHRONIC EFFECTS OF TEPOXALIN ON RENAL FUNCTION IN DOGS WITH CHRONIC KIDNEY DISEASE AND OSTEOARTHRITIS** |
| 11:30 am | NU-9 | Todd Archer | ULTRASOUND-GUIDED PLACEMENT OF URETHRAL STENTS IN DOGS |
| 11:45 am | NU-10 | Carrie Palm | URINARY NGAL: A BIOMARKER FOR EARLY IDENTIFICATION OF ACUTE KIDNEY INJURY IN DOGS |
| **12:00 pm** | **NU-11** | **Winnie Ybarra** | **ACCURACY OF URICULT® VET PADDLES FOR THE DIAGNOSIS AND IDENTIFICATION OF BACTERIAL CYSTITIS IN DOGS AND CATS** |
| 12:15 pm | NU-12 | Mary Thompson | ASYMPTOMATIC BACTERIURIA ESCHERICHIA COLI STRAIN 83972 IN COMPETITION WITH EMERGING, HIGHLY VIRULENT MULTI-DRUG RESISTANT *ESCHERICHIA COLI* STRAINS IN CANINE URINE |

**SMALL ANIMAL – INFECTIOUS DISEASE****

| | | | |
|---|---|---|---|
| 9:00 am | ID-1 | Megan Downey | *CYTAUXZOON FELIS* CYTOCHROME *B* GENOTYPE IS ASSOCIATED WITH SURVIVAL IN DOMESTIC CATS WITH CYTAUXZOONOSIS |
| 9:15 am | ID-2 | Leah Cohn | DIAMENAZINE DIACETURATE AT 3 MG/KG DOES NOT ELIMINATE PARASITEMIA IN CATS WITH CHRONIC *CYTAUXZOON FELIS* INFECTION |
| **9:30 am** | **ID-3** | **Audra Fenimore** | **DETECTION OF LEPTOSPIRURIA IN SHELTER CATS IN COLORADO** |
| 9:45 am | ID-4 | Matthew Krecic | IDENTIFICATION OF RHOPTRY ASSOCIATED PROTEIN-1S (RAP-1S) WITH BABSESIA GIBSONI ISOLATES OF INFECTED DOGS IN THE UNITED STATES |
| *BREAK* | | | |
| 10:30 am | ID-5 | Paolo Silvestrini | IRON STATUS AND C-REACTIVE PROTEIN IN CANINE LEISHMANIASIS |
| 10:45 am | ID-6 | Craig Ruaux | PREVALENCE OF *BLASTOCYSTIS* SPP AND *GIARDIA* SPP IN DOGS AND CATS RESIDENT IN A PACIFIC NORTHWEST SHELTER |
| **11:00 am** | **ID-7** | **Audra Fenimore** | **TREATMENT OF CHRONIC RHINITIS IN SHELTER CATS WITH PARENTERAL ALPHA-INTERFERON OR AN INTRANASAL FELINE HERPESVIRUS 1 AND FELINE CALICIVIRUS VACCINE** |
| **11:15 am** | **ID-8** | **Allison Bradley** | **INTRANASAL ADMINISTRATION OF A MODIFIED LIVE FELINE HERPESVIRUS 1 AND FELINE CALICIVIRUS VACCINE INDUCES CROSS PROTECTION AGAINST *BORDETELLA BRONCHISEPTICA*** |
| 11:30 am | ID-9 | Christian Leutenegger | FELINE HERPESVIRUS 1 VIRAL LOAD AS A TOOL TO DIFFERENTIATE LYTIC FROM LATENT INFECTION |
| 11:45 am | ID-10 | Annette Litster | SERIAL FIV SEROLOGICAL RESULTS IN COHABITING FIV-POSITIVE AND FIV-NEGATIVE CATS |
| 12:00 pm | ID-11 | Robert Lavan | U. S. PREVALENCE OF CANINE INFECTIOUS RESPIRATORY DISEASE PATHOGENS: A 3-YEAR STUDY |
| 12:15 pm | ID-12 | Linda Kidd | ARTHROPOD-BORNE DISEASE IN SICK SOUTHERN CALIFORNIA DOGS |

***Also see Infectious Disease abstracts ID-13 – ID-17 (Thursday, May 31, 2:45 pm – 4:00 pm)*

**EQUINE**

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|---|---|---|---|
| 9:00 am | E-1 | Colin Schwarzwald | ECHOCARDIOGRAPHIC ASSESSMENT OF LEFT VENTRICULAR SIZE AND SYSTOLIC FUNCTION IN HORSES USING LINEAR MEASUREMENTS AND AREA-BASED VOLUME ESTIMATES |

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| **9:15 am** | **E-2** | **Mark Bowen** | **VALIDATION OF A POINT OF CARE ULTRASOUND DEVICE FOR THE ASSESSMENT OF CARDIAC DISEASE IN THE HORSE** |
| **9:30 am** | **E-3** | **Erin McConachie** | **DOPPLER AND VOLUMETRIC ECHOCARDIOGRAPHIC METHODS FOR CARDIAC OUTPUT MEASUREMENT IN STANDING ADULT HORSES** |
| 9:45 am | E-4 | Stacy Tinkler | EFFECTS OF PRE-RACE FUROSEMIDE ADMINISTRATION ON PLASMA ELECTROLYTE CONCENTRATIONS AND ACID-BASE BALANCE IN STANDARDBREDS UNDERGOING A SIMULATED RACE PROTOCOL |
| *BREAK* | | | |
| 10:30 am | E-5 | Stacey Sullivan | ASSOCIATION BETWEEN EXERCISE-INDUCED PULMONARY HAEMORRHAGE AND DURATION OF RACING CAREER IN THOROUGHBRED RACE HORSES |
| 10:45 am | E-6 | Maty Looijen | HYPERBARIC OXYGEN THERAPY DOES NOT ALTER mRNA INFLAMMATORY GENES EXPRESSION IN HEALTHY HORSES |
| **11:00 am** | **E-7** | **Krista Estell** | **TEMPORAL VIRAL LOAD IN SEVEN HORSES WITH NATURALLY OCCURRING EQUINE HERPESVIRUS-1 MYELOENCEPHALOPATHY** |
| 11:15 am | E-8 | Nicola Pusterla | INVESTIGATION OF THE ROLE OF MULES DURING AN OUTBREAK OF EQUINE HERPESVIRUS-1 MYELOENCEPHALOPATHY IN CALIFORNIA |
| 11:30 am | E-9 | Nathan Slovis | EQUINE SALMONELLOSIS. THE USE OF AN ENHANCED RAPID TEST SYSTEM (Reveal® 2.0 Salmonella Test System) FOR EARLY DETECTION OF *SALMONELLA* IN FECES AND ENVIRONMENTAL SAMPLES |
| 11:45 am | E-10 | Gillian Perkins | ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* ISOLATES OBTAINED FROM HORSES IN THE NORTHEASTERN UNITED STATES |
| **12:00 pm** | **E-11** | **Florence Polle** | **ROLE OF INTRAOCULAR BACTERIAL INFECTIONS IN HORSES WITH RECURRENT UVEITIS IN LOUISIANA** |
| **12:15 pm** | **E-12** | **Raffaella Teixeira** | **GENETIC DETERMINANTS OF MELANOMA SUSCEPTIBILITY IN GRAY HORSES** |

**SMALL ANIMAL – CARDIOLOGY****

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|---|---|---|---|
| 2:15 pm | C-13 | Manuela Perego | ANALYSIS OF ATRIAL REPOLARIZATION WAVE ($T_a$) IN DOGS WITH THIRD-DEGREE ATRIOVENTRICULAR BLOCK |
| 2:30 pm | C-14 | Jorge Vila | STRUCTURAL AND MOLECULAR PATHOLOGY OF THE ATRIUM IN BOXER ARRHYTHMOGENIC CARDIOMYOPATHY |
| 2:45 pm | C-15 | Jordan Vitt | INCIDENCE OF HEART DISEASE IN CATS WITH ELECTROCARDIOGRAPHIC EVIDENCE OF LEFTWARD MEAN ELECTRICAL AXIS SHIFT |
| **3:00 pm** | **C-16** | **Jennifer Mulz** | **NT-PROBNP AS A SCREENING TOOL TO DETECT CLINICALLY SIGNIFICANT HEART DISEASE IN ASYMPTOMATIC CATS WITH HEART MURMURS AND/OR GALLOP RHYTHMS** |
| **3:15 pm** | **C-17** | **Bethany Smouther** | **HEART RATE AND ARRHYTHMIA FREQUENCY OF NORMAL CATS COMPARED TO CATS WITH HYPERTROPHIC CARDIOMYOPATHY** |
| **3:30 pm** | **C-18** | **Bethany Smouther** | **EFFECT OF ATENOLOL ON HEART RATE, ARRHYTHMIAS, BLOOD PRESSURE, AND DYNAMIC LEFT VENTRICULAR OUTFLOW TRACT OBSTRUCTION IN CATS WITH ASYMPTOMATIC HYPERTROPHIC CARDIOMYOPATHY** |
| 3:45 pm | C-19 | Andrea Lantis | THE EFFECT OF ENALAPRIL ON FUROSEMIDE-ACTIVATED RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS) IN NORMAL DOGS |

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| **4:30 pm** | **C-20** | **Marisa Ames** | **THE EFFECT OF HIGH DOSE PIMOBENDAN ON THE FUROSEMIDE-INDUCED RENIN-ANGIOTENSIN-ALDOSTERONE-SYSTEM (RAAS)** |
| **4:45 pm** | **C-21** | **Mari Waterman** | **DURATION OF BETA-BLOCKADE ASSOCIATED WITH REPEATED ONCE-DAILY ADMINISTRATION OF ATENOLOL IN HEALTHY DOGS.** |
| **5:00 pm** | **C-22** | **Dennis Trafny** | **EFFECT OF KETANSERIN, A SEROTONIN 2A-RECEPTOR ANTAGONIST, ON ECHOCARDIOGRAPHIC INDICES OF MITRAL REGURGITATION IN DOGS WITH DEGENERATIVE MITRAL VALVE DISEASE** |
| 5:15 pm | C-23 | Sonja Fonfara | MYOCARDIAL ICAM1 mRNA IS INCREASED IN CANINE DILATED CARDIOMYOPATHY |
| 5:30 pm | C-24 | Melanie Hezzell | ASSOCIATIONS BETWEEN N-TERMINAL PROCOLLAGEN TYPE III (PIIINP), FIBROSIS AND ECHOCARDIOGRAPHIC INDICES IN DOGS WITH MITRAL VALVE DISEASE |
| **5:45 pm** | **C-25** | **Kristen Parker** | **NOT ALL ARTERIES ARE CREATED EQUAL: COMPARISON OF PRESSURES WITHIN THE AORTIC ROOT AND THE DORSAL PEDAL ARTERY IN CATS** |
| **6:00 pm** | **C-26** | **Brandon Pogue** | **RETROGRADE CORONARY VENOUS STEM CELL DELIVERY: A PILOT STUDY EVALUATING SAFETY AND FEASIBILITY** |

***Also see Cardiology abstracts C-1 – C-12 (Thursday, May 31, 9:00 am – 12:30 pm)*

**SMALL ANIMAL - HEMATOLOGY**

| | | | |
|---|---|---|---|
| 2:15 pm | HM-1 | Anna Dengate | THE OVERALL HAEMOSTASIS POTENTIAL; A NOVEL, COST EFFECTIVE, GLOBAL COAGULATION ASSAY FOR USE IN CANINE PLASMA |
| 2:30 pm | HM-2 | Reinhard Mischke | MEASUREMENT OF THROMBIN GENERATION IN DOGS WITH DIFFERENT DISEASES |
| **2:45 pm** | **HM-3** | **Bérénice Conversy** | ***IN VITRO* EVALUATION OF THE EFFECT OF RIVAROXABAN ON COAGULATION PARAMETERS IN HEALTHY DOGS** |
| **3:00 pm** | **HM-4** | **Fiona Park** | **ASSESSMENT OF HYPERCOAGULABILITY IN CANINE PITUITARY-DEPENDENT HYPERADRENOCORTICISM** |
| 3:15 pm | HM-5 | Anna Dengate | DELAYED FIBRINOLYSIS IN DOGS WITH NATURALLY OCCURRING PITUITARY DEPENDANT HYPERADRENOCORTICISM |
| 3:30 pm | HM-6 | Craig Ruaux | SYRINGE AND AGGREGATE FILTER ADMINISTRATION DOES NOT AFFECT SURVIVAL OF TRANSFUSED AUTOLOGOUS FELINE RED BLOOD CELLS |
| 3:45 pm | HM-7 | Jessica Schavone | LEUKOREDUCTION OF FELINE WHOLE BLOOD USING A NEONATAL LEUKOCYTE REDUCTION FILTER: A PILOT EVALUATION |

*BREAK*

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|---|---|---|---|
| 4:30 pm | HM-8 | Alex Lynch | UTILITY OF SERIAL ANTI-XA MONITORING IN DOGS RECEIVING DALTEPARIN |
| 4:45 pm | HM-9 | Reinhard Mischke | PLATELET FUNCTION IN DOGS WITH INFLAMMATORY DISEASES |
| 5:00 pm | HM-10 | Alice Defarges | 15-GAUGE CORE BONE MARROW BIOPSY IN SMALL DOGS AND CATS WITH HEMATOLOGIC DISORDERS – 16 CASES |
| 5:15 pm | HM-11 | Stephanie Smith | SERUM BIOMARKERS INDICATE HEMOLYSIS AND AN INFLAMMATORY RESPONSE TO TRANSFUSION OF AUTOLOGOUS STORED ERYTHROCYTE CONCENTRATES |
| 5:30 pm | HM-12 | Sean Majoy | ASSESSMENT OF PLATELET ACTIVATION IN DOGS ADMITTED TO THE INTENSIVE CARE UNIT (ICU) BY USE OF FLOW CYTOMETRY AND THROMBOELASTOGRAPHY |

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| 5:45 pm | HM-13 | Melissa Bucknoff | EVALUATION OF THROMBOELASTOGRAPHY TO PREDICT CLINICAL BLEEDING IN THROMBOCYTOPENIC DOGS AFTER TOTAL BODY IRRADIATION AND HEMATOPOIETIC CELL TRANSPLANTATION |
| 6:00 pm | HM-14 | Andrea Zoia | IMMUNE-MEDIATED HEMOLYTIC ANEMIA IN CATS WITH PANCREATITIS |

**SMALL ANIMAL – NEUROLOGY / PHARMACOLOGY**

| | | | |
|---|---|---|---|
| **2:15 pm** | **N-1** | **Christopher Levine** | **CEREBELLAR ABIOTROPHY IN FOUR RELATED POMERANIANS** |
| 2:30 pm | N-2 | Unity Jeffery | DOES CEREBROSPINAL FLUID CYTOLOGY PREDICT CENTRAL NERVOUS SYSTEM HISTOLOGY? |
| **2:45 pm** | **N-3** | **Katherine Crook** | **THE PHARMACOKINETICS OF CYTARABINE IN DOGS WHEN ADMINISTERED VIA SUBCUTANEOUS AND CONTINUOUS INTRAVENOUS INFUSION ROUTES** |
| 3:00 pm | N-4 | Intan Shafie | CEREBROSPINAL FLUID CLUSTERIN IS A POTENTIAL BIOMARKER OF CANINE NEURODEGENERATIVE DISORDERS |
| 3:15 pm | N-5 | Daniel Sanchez-Masian | L-2-HYDROXYGLUTARIC ACIDURIA IN YORKSHIRE TERRIER DOGS: CHARACTERIZATION OF THE MOLECULAR DEFECT |
| **3:30 pm** | **N-6** | **Kendra Mikoloski** | **DIAGNOSTIC VALUE AND DISCRIMINATORY ABILITY OF PROTON MAGNETIC RESONANCE SPECTROSCOPY FOR INTRACRANIAL NEOPLASIA IN DOGS** |
| **3:45 pm** | **N-7** | **Cona Anwer** | **MAGNETIC RESONANCE IMAGING FEATURES OF INTRACRANIAL GRANULAR CELL TUMORS IN SEVEN DOGS** |
| *BREAK* | | | |
| **4:30 pm** | **N-8** | **Devon Hague** | **SIGNALMENT, CLINICAL FINDINGS, MAGNETIC RESONANCE IMAGING FEATURES AND SURGICAL OUTCOMES WITH HISTOPATHOLOGICALLY CONFIRMED EPIDURAL AND SUBDURAL SPINAL CORD HEMATOMA IN DOGS** |
| **4:45 pm** | **N-9** | **Jessica Barker** | **MRI ASSESSMENT OF DOGS WITH DESCENDING TRANSTENTORIAL AND / OR FORAMEN MAGNUM BRAIN HERNIATIONS** |
| **5:00 pm** | **N-10** | **Kari Foss** | **KINEMATIC GAIT ANALYSIS USING 3-D MOTION MOTION CAPTURE IN DOBERMAN PINSCHERS WITH AND WITHOUT CERVICAL SPONDYLOMYELOPATHY** |
| 5:15 pm | N-11 | Thomas Flegel | THE VACUUM PHENOMENON IN INTERVERTEBRAL DISC DISEASE OF DOGS BASED ON COMPUTED TOMOGRAPHY IMAGES |
| 5:30 pm | N-12 | E Beltran | MUTATION ASSOCIATED WITH NEONATAL CEREBELLAR CORTICAL DEGENERATION IN BEAGLE DOGS IDENTIFIED BY GENOME-WIDE MRNA SEQUENCING |
| **5:45 pm** | **N-13** | **Starr Cameron** | **SURGICAL REMOVAL OF FELINE INTRACRANIAL MENINGIOMAS: CLINICAL FEATURES AND OUTCOME IN 121 CASES (1994–2011)** |
| **6:00 pm** | **P-1** | **Michaela Beasley** | **THE PHARMACOKINETICS OF SINGLE DOSE EXTENDED RELEASE KEPPRA® WITH AND WITHOUT FOOD IN HEALTHY ADULT DOGS** |

**SMALL ANIMAL – RESPIRATORY / OTHER**

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|---|---|---|---|
| **2:15 pm** | **R-1** | **Katharine Woods** | **COMPARISON OF BRONCHOALVEOLAR LAVAGE ASPIRATION TECHNIQUES IN HEALTHY DOGS: MANUAL ASPIRATION VIA POLYETHYLENE TUBING AND SUCTION PUMP ASPIRATION** |
| 2:30 pm | R-2 | Richard Stone | ENVIRONMENTAL AIR QUALITY, METEOROLOGICAL FACTORS, AND FELINE RESPIRATORY DISEASE |
| 2:45 pm | R-3 | Erinne Branter | TRACHEAL MALFORMATIONS IN YORKSHIRE TERRIERS |

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|---|---|---|---|
| 3:00 pm | R-4 | Chee-hoon Chang | ORAL GLUCOCORTICOIDS MAY DIMINISH EFFICACY OF ALLERGEN-SPECIFIC IMMUNOTHERAPY IN EXPERIMENTAL FELINE ASTHMA |
| 3:15 pm | R-5 | Chee-hoon Chang | FELINE T REGULATORY CELLS IN BRONCHOALVEOLAR LAVAGE FLUID AND WHOLE BLOOD IN HEALTHY AND EXPERIMENTALLY ASTHMATIC CATS |
| 3:30 pm | R-6 | A. Ray Dillon | LUNG PATHOLOGY ASSOCIATED WITH TOXOCARA CATI INFECTION IN CATS IS INDEPENDENT OF DEVELOPMENT OF ADULT INTESTIONAL PARASITES |
| 3:45 pm | R-7 | Laura Nafe | CERVICAL LUNG LOBE HERNIATION IN DOGS WITH COUGH AS IDENTIFIED BY FLUOROSCOPY |
| *BREAK* | | | |
| 4:30 pm | R-8 | Yukihito Shiroshita | ASSESSMENT OF ARTERIALIZATION OF PERIPHERAL VENOUS BLOOD IN WELL-PERFUSED DOGS |
| 4:45 pm | OT-1 | | Withdrawn |
| 5:00 pm | OT-2 | Alexandra Floras | HOSPITAL VERSUS COMMUNITY ACQUIRED INTRA-ABDOMINAL INFECTION OF GASTROINTESTINAL ORIGIN IN DOGS |
| 5:15 pm | OT-3 | Medora Pashmakova | MULTI-CENTER EVALUATION OF THE ADMINISTRATION OF CROTALID ANTIVENOM IN CATS |
| **5:30 pm** | **OT-4** | **Stacy Burdick** | **ENDOSCOPIC-GUIDED LASER ABLATION OF VESTIBULOVAGINAL DEFECTS IN 36 DOGS** |
| 5:45 pm | OT-5 | Joshua Rowe | $^{18}$FLT-PET/CT FOR NON-INVASIVE FUNCTIONAL IMAGING OF CANINE BONE MARROW |
| 6:00 pm | OT-6 | Kara Osterbur | LIPOPOLYSACCHARIDE, TUMOR NECROSIS FACTOR, AND INTERLEUKIN-1β INDUCE NT-PRO C-TYPE NATRIUETIC PEPTIDE SECRETION FROM CANINE VASCULAR ENDOTHELIAL CELLS |

**SMALL ANIMAL - GASTROENTEROLOGY****

| | | | |
|---|---|---|---|
| 2:15 pm | GI-1 | Melissa Markel | CHARACTERIZATION OF FECAL DYSBIOSIS IN DOGS WITH CHRONIC ENTEROPATHIES AND ACUTE HEMORRHAGIC DIARRHEA |
| 2:30 pm | GI-2 | Maria Volkmann | FINAL DIAGNOSES IN 136 DOGS WITH CHRONIC DIARRHEA |
| 2:45 pm | GI-3 | Niels Grützner | SERUM HOMOCYSTEINE CONCENTRATIONS IN CHINESE SHAR PEIS AND DOGS OF SIX OTHER BREEDS WITH COBALAMIN DEFICIENCY |
| 3:00 pm | GI-4 | Caroline Mansfield | CONFOCAL ENDOMICROSCOPY ENABLES *IN VIVO* IDENTIFICATION OF GASTRIC HELICOBACTER-LIKE ORGANISMS IN DOGS |
| 3:15 pm | GI-5 | Romy Heilmann | FECAL S100A12 CONCENTRATIONS ARE INCREASED IN DOGS WITH INFLAMMATORY BOWEL DISEASE |
| 3:30 pm | GI-6 | Katie Stroda | HISTOPATHOLOGICAL, CLINICAL, ENDOSCOPIC, AND ULTRASOUND FEATURES OF DOGS WITH CHRONIC ENTEROPATHIES AND SMALL INTESTINAL CRYPT LESIONS |
| 3:45 pm | GI-7 | Itamar Aroch | SERUM PEPSINOGEN A AS A PROGNOSTIC MARKER IN CANINE GASTRIC DILATATION AND VOLVULUS |

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| **4:45 pm** | **GI-8** | **Caroline Goutal** | **COMPARISON OF QUALITY AND DIAGNOSTIC ADEQUACY OF CANINE DUODENAL MUCOSAL BIOPSY SPECIMENS OBTAINED WITH DIFFERENT ENDOSCOPIC FORCEPS IN 17 DOGS** |
| **5:00 pm** | **GI-9** | **Cris Otoni** | **SEROLOGIC AND FECAL MARKERS IN PREDICTION OF ACUTE DISEASE COURSE IN CANINE CHRONIC ENTEROPATHIES** |
| 5:15 pm | GI-10 | Alexandra Galler | FLOW CYTOMETRICAL ANALYSIS OF PERIPHERAL BLOOD MONONUCLEAR CELLS IN DOGS WITH INFLAMMATORY BOWEL DISEASE |
| 5:30 pm | GI-11 | Shingo Maeda | DECREASED IMMUNOGLOBULIN A LEVELS IN FECES, DUODENUM, AND PERIPHERAL BLOOD LYMPHOCYTES OF DOGS WITH INFLAMMATORY BOWEL DISEASE |
| 5:45 pm | GI-12 | Panagiotis Xenoulis | FELINE EXOCRINE PANCREATIC INSUFFICIENCY: 150 CASES |
| 6:00 pm | GI-13 | Katie Tolbert | EXOGENOUS SIALIC ACID AND CYSTEINE PROTEASE INHIBITION BLOCK ADHERENCE OF *TRITRICHOMONAS FOETUS* TO THE INTESTINAL EPITHELIUM |

***Also see Gastroenterology abstracts GI-14 – GI-16 (Friday, June 1, 9:15 am – 10:00 am).*

**SMALL ANIMAL – IMMUNOLOGY / INFECTIOUS DISEASE** / ONCOLOGY**

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| **2:15 pm** | **IM-1** | **Andrea Wang** | **EFFICACY OF MYCOPHENOLATE MOFETIL FOR THE TREATMENT OF CANINE IMMUNE-MEDIATED HEMOLYTIC ANEMIA: 31 CASES (2007-2011)** |
| **2:30 pm** | **IM-2** | **Laura Nafe** | ***EX VIVO* IMMUNOSUPPRESSION OF CANINE T LYMPHOCYTE-SPECIFIC PROLIFERATION USING DEXAMETHASONE, CYCLOSPORINE, AND THE ACTIVE METABOLITES OF AZATHIOPRINE AND LEFLUNOMIDE IN A FLOW CYTOMETRIC ASSAY** |
| 2:45 pm | ID-13 | Nahvid Etedali | NT-pCNP AS A DIAGNOSTIC BIOMARKER FOR SEPSIS IN DOGS PRESENTING FOR EMERGENCY CARE |
| 3:00 pm | ID-14 | Monika Burns | SERIAL NT-pCNP in HOSPITALIZED DOGS WITH SEPSIS |
| **3:15 pm** | **ID-15** | **Amy Davenport** | **PHYLOGENETIC DIVERSITY OF BACTERIA ISOLATED FROM SICK DOGS USING THE BAPGM ENRICHMENT CULTURE PLATFORM** |
| **3:30 pm** | **ID-16** | **Kimberly Yore** | **PREVALENCE OF *BARTONELLA* SPP. AND HEMOPLASMAS IN THE BLOOD OF DOGS AND THEIR FLEAS IN FLORIDA** |
| **3:45 pm** | **ID-17** | **Lindsay Tangeman** | **CLINICOPATHOLOGIC FEATURES AND ATYPICAL PRESENTATIONS OF NATURALLY OCCURRING CANINE LEPTOSPIROSIS: 51 CASES (2000-2010)** |

*** Also see Infectious Disease abstracts ID-1 – ID-12 (Thursday, May 31, 9:00 am – 12:30 pm)*

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| 4:30 pm | O-1 | Esther Chon | CHARACTERIZATION OF BETA-CATENIN EXPRESSION IN CANINE ORAL MELANOMA (VCS AWARD WINNER) |
| 4:45 pm | O-2 | Lindsay Thalheim | AGREEMENT BETWEEN FLOW CYTOMETRY, PCR FOR ANTIGEN RECEPTOR REARRANGEMENT, AND IMMUNOHISTOCHEMISTRY IN CANINE LYMPHOMA (VCS AWARD WINNER) |
| 5:00 pm | O-3 | Deborah Knapp | FIROCOXIB HAS ANTITUMOR ACTIVITY AS A SINGLE AGENT AND ENHANCES THE ACTIVITY OF CISPLATIN IN DOGS WITH TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER |
| **5:15 pm** | **O-4** | **Mitchell Kaye** | **PHASE II CLINICAL TRIAL OF VINORELBINE AND PIROXICAM FOR DOGS WITH TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER** |

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| 5:30 pm | O-5 | Krysta Deitz | COMPUTED TOMOGRAPHIC APPEARANCE OF CANINE THYROID TUMORS |
| 5:45 pm | O-6 | Cecilia Robat | MULTI-INSTITUTION RETROSPECTIVE STUDY OF 85 CASES OF CANINE THYMOMA (1999-2010) |
| 6:00 pm | O-7 | Krysta Deitz | EVALUATION OF SECRETED FRIZZLED-RELATED PROTEIN 2 EXPRESSION IN CANINE THYROID TUMORS USING RT-PCR AND IMMUNOHISTOCHEMISTRY |

**EQUINE**

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| **2:15 pm** | **E-13** | **Victoria Scott** | **EFFECT OF BODY POSITION ON ABDOMINAL PRESSURES IN ADULT HORSES** |
| 2:30 pm | E-14 | Harold Schott II | VOLUME OF DISTENDED SMALL INTESTINE AND IONIC COMPSITION OF GASTRIC REFLUX AND SMALL INTESTINAL FLUID IN HORSES |
| 2:45 pm | E-15 | Jocelyn Habershon-Butcher | PREVALENCE AND RISK FACTORS FOR ULCERATION OF THE GASTRIC GLANDULAR MUCOSA IN THOROUGHBRED RACEHORSES IN TRAINING IN THE UK AND AUSTRALIA |
| 3:00 pm | E-16 | James Belknap | REGULATION OF HYPOXIA INDUCIBLE FACTOR-1A AND RELATED GENES IN THE EQUINE KERATINOCYTE AND DIGITAL LAMINAE IN EXPERIMENTAL MODELS OF EQUINE LAMINITIS |
| 3:15 pm | E-17 | Teresa Burns | LAMINAR INFLAMMATORY EVENTS IN LEAN AND OBESE PONIES SUBJECTED TO HIGH CARBOHYDRATE FEEDING: IMPLICATIONS FOR PASTURE-ASSOCIATED LAMINITIS |
| 3:30 pm | E-18 | Heidi Banse | ABSENCE OF OXIDATIVE STRESS IN THE SKELETAL MUSCLE OF OBESE HORSES |
| **3:45 pm** | **E-19** | **Kate McGovern** | **THE EFFECTS OF EXPERIMENTALLY INDUCED HYPERGLYCEMIA AND ENDOTOXEMIA ON COAGULATION PARAMETERS IN HEALTHY ADULT HORSES** |
| *BREAK* | | | |
| 4:30 pm | E-20 | Jennifer Mahon | EVALUATION OF HYPERCOAGULABILTY IN GERIATRIC HORSES WITH/WITHOUT PITUITARY PARS INTERMEDIA DYSFUNCTION |
| **4:45 pm** | **E-21** | **Katarzyna Dembek** | **RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS) RESPONSE AND ACTH/ ALDOSTERONE RATIO IN SEPTIC NEWBORN FOALS** |
| 5:00 pm | E-22 | Martin Furr | EFFECTS OF BLOOD CONTAMINATION ON PARAMETERS OF CEREBROSPINAL FLUID ANALYSIS |
| **5:15 pm** | **E-23** | **Alexandra Draper** | **THE EPIDEMIOLOGY OF SHIVERS IN HORSES** |
| 5:30 pm | E-24 | Nora Nogradi | PROGNOSTIC FACTORS ASSOCIATED WITH SHORT-TERM AND LONG-TERM OUTCOME IN HORSES WITH CHRONIC RENAL FAILURE |
| 5:45 pm | E-25 | Noah Cohen | *IN VIVO* ADMINISTRATION OF CPG ENHANCES FOAL NEUTROPHIL FUNCTION |
| 6:00 pm | E-26 | Alexandra Burton | EFFECT OF AGE ON THE PHARMACOKINETICS OF A SINGLE DAILY DOSE OF GENTAMICIN SULPHATE IN FOALS |

**FOOD ANIMAL**

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|---|---|---|---|
| 2:15 pm | FA-1 | Christopher Luby | SKILLS REQUIRED OF ENTRY LEVEL VETERINARIANS: A SURVEY OF DAIRY PRACTITIONERS |
| 2:30 pm | FA-2 | Scott Weese | METAGENOMIC EVALUATION OF THE INTESTINAL MICROBIOME IN VEAL CALVES AND THE INFLUENCE OF ORAL TETRACYCLINE |
| 2:45 pm | FA-3 | Scott Weese | A PROSPECTIVE CONTROLLED STUDY ON PREVALENCE OF *CLOSTRIDIUM DIFFICILE* IN VEAL CALVES RECEIVING AND NOT RECEIVING ORAL OXYTETRACYCLINE |

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| 3:00 pm | FA-4 | Angelika Schoster | INCREASED STRONG ION GAP IN DAIRY CALVES WITH DIARRHEA IS LIKELY DUE TO D-LACTATE |
| 3:15 pm | FA-5 | Gillian Perkins | ANTIMICROBIAL RESISTANCE TRENDS AMONG *SALMONELLA* ISOLATES OBTAINED FROM DAIRY CATTLE IN THE NORTHEASTERN USA, 2004-2010 |
| **3:30 pm** | **FA-6** | **Gretchen Grissett** | **EVALUATION OF ORALLY SUPPLEMENTED D, L-METHIONINE AS A URINE ACIDIFIER FOR SMALL RUMINANTS** |
| **3:45 pm** | **FA-7** | **Philippa Sprake** | **THE EFFECT OF AMMONIUM CHLORIDE TREATMENT AS A LONG TERM PREVENTATIVE APPROACH FOR UROLITHIASIS IN GOATS AND A COMPARISON OF CONTINUOUS AND PULSE DOSING REGIMES** |
| *BREAK* | | | |
| 4:30 pm | FA-8 | Sébastien Buczinski | ECHOCARDIOGRAPHIC MEASUREMENT OF VALVULAR THICKNESS IN HEALTHY COWS, COWS WITH BACTERIAL ENDOCARDITIS AND COWS WITH CARDIORESPIRATORY DISEASES |
| 4:45 pm | FA-9 | | Withdrawn |
| 5:00 pm | FA-10 | Meera Heller | UTERINE NEOPLASIA IN SMALL RUMINANTS: RETROSPECTIVE STUDY (1991-2011) |
| **5:15 pm** | **FA-11** | **Piper Norton** | **CAMELID HEAT STRESS AS A SYNDROME: 15 CASES (2003–2011)** |
| **5:30 pm** | **FA-12** | **Toby Pinn** | **COMPARISON OF THREE IMMUNOGLOBULIN G (IgG) ASSAYS FOR DIAGNOSIS OF FAILURE OF PASSIVE TRANSFER IN NEONATAL ALPACAS** |
| **5:45 pm** | **FA-13** | **Amanda Kreuder** | **BIOAVAILABILITY AND PHARMACOKINETICS OF ORAL MELOXICAM IN LLAMAS** |
| 6:00 pm | FA-14 | Kristen Thane | FLORFENICOL PHARMACOKINETICS IN HEALTHY ADULT ALPACAS, EVALUATING TWO COMMERCIALLY AVAILABLE DRUG FORMULATIONS |

# ORAL PRESENTATIONS – FRIDAY, JUNE 1

**SMALL ANIMAL – NUTRITION / METABOLISM**

| | | | |
|---|---|---|---|
| 8:00 am | NM-1 | Ebenezer Satyaraj | NUTRITIONAL IMMUNOMODULATION: BOVINE COLOSTRUM FOR IMMUNE & GUT HEALTH IN DOGS |
| 8:15 am | NM-2 | Alexander German | EFFECTS OF WEIGHT LOSS IN OBESE DOGS ON A RANGE OF RENAL BIOMARKERS |
| 8:30 am | NM-3 | Hannelore Van de Velde | mRNA EXPRESSION RESPONSE OF ADIPOKINES TO FELINE OBESITY DEPENDS ON ADIPOSE TISSUE LOCATION |
| 8:45 am | NM-4 | Isabelle Jeusette | COMPARISON OF TWO WEIGHT LOSS DIETS IN OVERWEIGHT CATS |
| 9:00 am | NM-5 | Angela Witzel (Lusby) | COMPARISION OF HIGH FIBER AND LOW CARBOHYDRATE DIETS ON OWNER-PERCEIVED SATIETY OF CATS DURING WEIGHT LOSS |
| 9:15 am | NM-6 | Samuel Serisier | SEASON AND MONTH EFFECTS ON FOOD INTAKE IN ADULT COLONY CATS |
| 9:30 am | NM-7 | Raphaël Goudez | INFLUENCE OF DIFFERENT SOURCES OF CORN STARCH ON STOOLS CHARACTERISTICS IN DOGS DIFFERING IN BODY SIZE |
| 9:45 am | NM-8 | Samuel Serisier | A 8.5-YEAR LONGITUDINAL STUDY TO IDENTIFY RISK FACTORS OF OBESITY IN COLONY CATS |

**SMALL ANIMAL – HEPATOLOGY / GASTROENTEROLOGY****

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|---|---|---|---|
| 8:00 am | HP-1 | Clémence Peyron | LIVER HISTOLOGICAL LESIONS IN 43 SCOTTISH TERRIERS (ST) WITH HYPERACTIVITY OF ALKALINE PHOSPHATASES (AP) |
| 8:15 am | HP-2 | Jonathan Lidbury | CLINICAL FEATURES OF HEPATIC ENCEPHALOPATHY IN DOGS: 80 CASES (1991–2011) |
| 8:30 am | HP-3 | Sharon Center | LONG-TERM SURVIVAL OF DOGS (n = 597) WITH CONGENITAL OR ACQUIRED PORTOSYSTEMIC SHUNTING: 1980-2010 |
| 8:45:am | HP-4 | Manabu Sakai | SERUM BRANCHED-CHAIN AMINO ACID/TYROSINE RATIO (BTR) IN DOGS WITH LIVER DISEASES |
| 9:00 am | HP-5 | Yumi Sakamoto | PLASMA AND HEPATIC ENDOTHELIN-1 IN DOGS WITH CHRONIC LIVER DISEASES |
| 9:15 am | GI-14 | Belgin Dogan | COMPARISON OF *E. COLI* ASSOCIATED WITH GRANULOMATOUS COLITIS OF BOXER DOGS AND *E. COLI* FROM HEALTHY CONROLS |
| 9:30 am | GI-15 | Alison Manchester | GRANULOMATOUS COLITIS IN FRENCH BULLDOGS IS ASSOCIATED WITH INVASIVE *E. COLI* AND CLINICAL RESPONSE TO FLUOROQUINOLONE ANTIBIOTICS |
| 9:45 am | GI-16 | Sabrina Hoehne | FELINE ALIMENTARY LYMPHOMA IS ASSOCIATED WITH CHANGES IN THE SPATIAL DISTRIBUTION OF MUCOSAL BACTERIA |

***Also see Gastroenterology abstracts GI-1 – GI-13 (Thursday, May 31, 2:15 pm – 6:15 pm).*

## POSTER PRESENTATIONS

On Display: Thursday, May 31, 7:00 am – 7:00 pm
Friday, June 1, 7:00 am – 7:00 pm
Saturday, June 2, 7:00 am – 2:30 pm

Attended by Authors Eligible for ACVIM Resident Research Awards:
Thursday, May 31, 9:50 am – 10:30 am
Friday, June 1, 9:50 am – 10:30 am

Attended by all Authors – Wine & Cheese Reception:
Friday, June 1, 6:00 pm – 7:30 pm

| # | Presenting Author | Abstract Title |
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**SMALL ANIMAL - CARDIOLOGY**

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| C-27 | David McGahie | THE NATURAL ANTIOXIDANTS POMEGRANATE EXTRACT AND SOY ISOFLAVONES FAVOURABLY MODULATE CANINE ENDOTHELIAL CELL FUNCTION |
| C-28 | David McGahie | POMEGRANATE EXTRACT COMBINED WITH OTHER NATURAL ANTIOXIDANTS PROTECTS CANINE ENDOTHELIAL CELLS FROM DEATH INDUCED BY OXIDATIVE STRESS |
| C-29 | Junhwan Kim | CHANGES OF SERUM CARDIAC TROPONIN I CONCENTRATION IN 13 DOGS WITH INTRACRANIAL DISORDERS |
| C-30 | Aleksandra Domanjko Petrič | C-REACTIVE PROTEIN IS ELEVATED IN DOGS IN SEVERE HEART FAILURE |
| C-31 | Hung-Yin Chen | ASSESSMENT OF HYPERTROPHIC CARDIOMYOPATHY IN SMALL BREED DOGS AFFECTED WITH HYPERADRENOCORTICISM USING 2D SPECKLE-TRACKING ECHOCARDIOGRAPHY |
| C-32 | Guilherme Goldfeder | ECHOCARDIOGRAPHIC EVALUATION OF LEFT ATRIAL SIZE IN HEALTH COCKER SPANIEL DOGS AND WITH DILATED CARDIOMIOPATHY |

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| C-33 | Antonio Camacho | TIME-DOMAIN HEART RATE VARIABILITY IN HEALTHY DOGS |
| C-34 | Maria Helena Larsson | HEART RATE VARIABILITY IN BOXER DOGS WITH ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY |
| C-35 | Joanelle Hernandez-Lopez | RADIOGRAPHIC VERTEBRAL HEART SIZE AND LEFT ATRIAL BISECTING LINE: INTEROBSERVER VARIABILITY AND COMPARISON TO ECHOCARDIOGRAPHIC LEFT ATRIAL SIZE IN DOGS WITH DEGENERATIVE MITRAL VALVE DISEASE |
| C-36 | Osuga Tatsuyuki | LEFT ATRIAL FUNCTION ANALYSIS BY LEFT ATRIAL TRACKING METHOD IN CANINE CHRONIC MITRAL VALVULAR HEART DISEASE |
| C-37 | Keisuke Sugimoto | EARLY DETECTION OF LEFT VENTRICULAR DYSFUNCTION IN DOGS WITH DOXORUBICIN-INDUCED CARDIOMYOPATHY BY 2D SPECKLE TRACKING ECHOCARDIOGRAPHY |
| C-38 | Antonio Camacho | DOXORUBICIN-INDUCED DILATED CARDIOMYOPATHY IN A RABBIT MODEL: AN UPDATE |
| C-39 | Maria Helena Larsson | ASSESSMENT OF SERUM ADIPONECTIN AND ITS CORRELATION WITH OBESITY AND MITRAL VALVE ENDOCARDIOSIS IN DOGS |
| C-40 | Antonio Camacho | ARTERIAL BLOOD PRESSURE ASSESSMENT IN NORMAL AND OVERWEIGHT DOGS BY THREE DIFFERENT METHODS |
| C-41 | Denise Schwartz | PLASMA LEVELS OF NT-PRO BNP AND VENTRICULAR DIASTOLIC FUNCTION ASSESSED BY PULSED TISSUE DOPPLER ECHOCARDIOGRAPHY IN OBESE DOGS |
| C-42 | Hung-Yin Chen | EFFECT OF BODYWEIGHT ON ASSESSMENT OF LEFT VENTRICULAR FUNCTIONS USING TWO-DIMENSIONAL SPECKLE-TRACKING ECHOCARDIOGRAPHY |
| C-43 | Denise Schwartz | ECHOCARDIOGRAPHIC FINDINGS IN OBESE DOGS |
| C-44 | Emily Mehlman | CHARACTERIZATION OF GROSS AND HISTOLOGIC MYOCARDIAL LESIONS IN OBESE DOGS |
| C-45 | Denise Schwartz | TRANSVENOUS PERMANENT PACEMAKER THERAPY IN DOGS: BRAZILIAN EXPERIENCE |
| C-46 | Paula Itikawa | ECHOCARDIOGRAPHIC EVALUATION OF LEFT ATRIAL SIZE IN MAINE COON CATS, FROM APICAL FOUR CHAMBER VIEW |
| **C-47** | **Gretchen Singletary** | **SURVIVAL OF CATS WITH ISOLATED DISCRETE UPPER SEPTAL THICKENING (DUST) VS. CATS WITH AND WITHOUT CARDIOMYOPATHY** |
| **C-48** | **Maggie Machen** | **EFFECT OF ATENOLOL THERAPY ON ECHOCARDIOGRAPHIC PARAMETERS IN CATS WITH HYPERTROPHIC OBSTRUCTIVE CARDIOMYOPATHY** |
| C-49 | Rachel Cohen | EFFECT OF A HEART MURMUR ON ADOPTABILITY OF CATS IN AN URBAN NO-KILL RESCUE ORGANIZATION |
| **C-50** | **Maggie Machen** | **DETECTION OF OCCULT FELINE CARDIOMYOPATHY USING A PET-SIDE POINT-OF-CARE NT-PROBNP ELISA ASSAY** |
| C-51 | Paula Itikawa | TRICUSPID ANNULAR PLANE SYSTOLIC EXCURSION (TAPSE) MEASUREMENTS IN MAINE COON CATS |
| C-52 | Guilherme Goldfeder | TRICUSPID ANNULAR PLANE SYSTOLIC EXCURSION (TAPSE) MEASUREMENTS IN DOGS |
| C-53 | Mark Oyama | SURVEY OF SIX POTENTIAL CARDIAC BIOMARKERS IN DOGS WITH HEART DISEASE: CHROMOGRANIN-A, ENDOGLIN, GALECTIN-3, NT-PROBNP, ST2, and OSTEOPONTIN |

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| C-54 | Ryan Birks | BREED-SPECIFIC VERTEBRAL HEART SIZE FOR THE DACHSHUND |
| C-55 | Anthony Carr | IN-HOME BLOOD PRESSURE MONITORING IN DOGS: A PILOT STUDY |
| **C-56** | **Damon Leeder** | **LONG-TERM SURVIVAL IN DOGS WITH UNTREATED SEVERE SUBAORTIC STENOSIS** |
| C-57 | Andrea Vollmar | COMPARISON OF PIMOBENDAN, BENAZEPRIL, OR METHYLDIGOXIN MONOTHERAPY IN IRISH WOLFHOUNDS WITH OCCULT DCM: A 10-YEAR-STUDY |

**SMALL ANIMAL - ENDOCRINOLOGY**

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| EN-13 | Ah Young Kim | EVALUATION OF ONCE-DAILY ADMINISTRATION OF INSULIN GLARGINE IN DOGS WITH NATURALLY OCCURING DIABETES MELLITUS |
| EN-14 | Marcia Jericó | CHROMATOGRAPHIC ANALYSIS OF THE LIPID FRACTIONS IN OBESE CLIENT-OWNED DOGS |
| EN-15 | Marcia Jericó | CHROMATOGRAPHIC ANALYSIS OF THE LIPID FRACTIONS IN OBESE CLIENT-OWNED CATS |

**SMALL ANIMAL - GASTROENTEROLOGY**

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| GI-17 | Dianne Mawby | NATURALLY-OCCURRING HYPERADRENOCORTICISM IS ASSOCIATED WITH INCREASED PANCREATIC LIPASE IMMUNOREACTIVITY CONCENTRATIONS IN DOGS |
| GI-18 | Jacqueline Whittemore | ALPHA-ENOLASE ANTIBODIES ARE NOT ASSOCIATED WITH FELINE PANCREATIC LIPASE IMMUNOREACTIVITY CONCENTRATIONS IN CLIENT-OWNED CAT |
| GI-19 | Hiroki Okanishi | MUCOSAL NUCLEOTIDE OLIGOMERIZATION DOMAIN TWO (NOD2) MRNA OVEREXPRESSION AND NF-KAPPAB ACTIVATION IN DOGS WITH CHRONIC ENTEROPATHY |
| GI-20 | Nora Berghoff | FECAL AND URINARY N-METHYLHISTAMINE CONCENTRATIONS IN DOGS WITH CHRONIC GASTROINTESTINAL DISEASE |
| GI-21 | Nora Berghoff | SERUM COBALAMIN AND METHYLMALONIC ACID CONCENTRATIONS IN DOGS WITH CHRONIC GASTROINTESTINAL DISEASE |
| GI-22 | Niels Grützner | SERUM CONCENTRATIONS OF CANINE ALPHA1-PROTEINASE INHIBITOR IN YORKSHIRE TERRIERS WITH AND WITHOUT COBALAMIN DEFICIENCY |
| GI-23 | Maria Volkmann | EFFECTIVITY OF AST-120 IN DOGS WITH CHRONIC INFLAMMATORY ENTEROPATHIES |
| GI-24 | Hiroshi Ohta | INTERLEUKIN-17A GENE EXPRESSION IN THE INTESTINAL MUCOSA IN DOGS WITH LYMPHOCYTIC-PLASMACYTIC ENTERITIS AND INFLAMMATORY COLORECTAL POLYP |
| GI-25 | Leda Barros | C-REACTIVE PROTEIN CONCENTRATIONS IN DOGS WITH INFLAMMATORY BOWEL DISEASE AND CORRELATION WITH A CLINICAL DISEASE ACTIVITY INDEX AND SERUM ALBUMIN CONCENTRATIONS |
| GI-26 | Craig Webb | THE EFFECT OF A MULTI-STRAIN SYNBIOTIC ON THE MICROBIOTA OF CATS WITH CHRONIC ENTERITIS |
| GI-27 | Alexis Lecoindre | REGIONAL GRANULOMATOUS ENTERITIS IN 14 DOGS. |
| GI-28 | Yasushi Minamoto | EVALUATION OF FECAL SHORT CHAIN FATTY ACID CONCENTRATIONS IN HEALTHY DOGS AND DOGS WITH CHRONIC DIARRHEA |
| GI-29 | Brittany Marvel | THE EFFECT OF VARIOUS STORAGE CONDITIONS ON THE ABUNDANCE OF BACTERIAL GROUPS IN CANINE FECES |

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| GI-30 | Jose Garcia-Mazcorro | DAY-TO-DAY VARIATION OF FECAL MICROBIOTA IN DOGS OF DIFFERENT AGES |
| GI-31 | Kohtaro Hayashi | PRESURGICAL EVALUATION OF RECTAL MASSES IN DOGS BY ENDOSCOPY AND ENDOSCOPIC ULTRASOUND: 28 CASES |

**SMALL ANIMAL - HEMATOLOGY**

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| HM-15 | Craig Webb | OSMOTIC FRAGILITY AND FLOW CYTOMETRIC DETERMINATION OF LIPID PEROXIDATION IN FELINE ERYTHROCYTES |
| HM-16 | Alice Defarges | POWER-DRIVER ASSISTED 11-GAUGE HUMERAL CORE BONE MARROW BIOPSY IN LARGE BREED DOGS WITH HEMATOLOGIC DISORDERS – 6 CASES |
| HM-17 | Haruhiko Maruyama | EVALUATION OF A HUMAN ELISA KIT FOR THE MEASUREMENT OF PLASMA ADAMTS13 ACTIVITY IN DOGS |

**SMALL ANIMAL – INFECTIOUS DISEASE**

| | | |
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| ID-18 | Leah Cohn | PARASITE BURDEN DOES NOT FLUCTUATE WITH TIME OF DAY IN CATS WITH CHRONIC *CYTAUXZOON FELIS* INFECTION |
| ID-19 | Megan Downey | MITOCHONDRIAL GENOME SEQUENCES RESULT IN IMPROVED UNDERSTANDING OF THE PHYLOGENETIC RELATIONSHIPS OF PIROPLASMIDA THAT INFECT COMPANION ANIMALS |
| ID-20 | Sukullaya Assarasakorn | PREVALENCE OF *BARTONELLA* SPP. ASSOCIATED CANINE INFECTIOUS ENDOCARDITIS IN BANGKOK, THAILAND |
| ID-21 | Rosalind Carslake | PREVALENCE OF SELECTED INFECTIOUS DISEASES IN SAMOAN DOGS |
| ID-22 | Jane Sykes | MOLECULAR EPIDEMIOLOGY AND ANTIFUNGAL SUSCEPTIBILITY AMONG CRYPTOCOCCUS ISOLATES FROM NORTH AMERICAN DOGS AND CATS |
| ID-23 | Michael Lappin | DEVELOPMENT OF AN INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF FELINE ANTIBODIES AGAINST *MYCOPLASMA FELIS* |
| ID-24 | Michael Lappin | DETECTION OF FELINE ANTIBODIES AGAINST A NOVEL *ANAPLASMA PHAGOCYTOPHILUM* PEPTIDE (AP-4) AFTER EXPOSURE TO WILD-CAUGHT ADULT *IXODES SCAPULARIS* |
| ID-25 | Scott Moroff | DETECTION OF CANINE SERUM ANTIBODIES AGAINST A NOVEL MUTANT PEPTIDE OF *ANAPLASMA PHAGOCYTOPHILUM* |
| ID-26 | Michael Lappin | VACCINE-ASSOCIATED *LEPTOSPIRA* ANTIBODY RESPONSES IN CLIENT-OWNED DOGS |
| ID-27 | Jhoanna Rodriguez | FELINE LEPTOSPIROSIS: A SEROLOGIC AND URINARY PCR SURVEY IN HEALTHY CATS AND IN CATS WITH KIDNEY DISEASE |
| ID-28 | Kuribayashi Hagiwara | ISOLATION AND CHARACTERIZATION OF *LEPTOSPIRA INTERROGANS* SEROVAR COPENHAGENI AND SEROVAR CANICOLA FROM DOGS WITH LEPTOSPIROSIS |
| ID-29 | Emily Barker | NON-RIBOSOMAL GENE PHYLOGENY OF HEMOPLASMAS AND OTHER *MYCOPLASMA* SPECIES |
| ID-30 | Michael Lappin | FAILURE TO AMPLIFY *BARTONELLA KOEHLERAE* DNA FROM BLOOD OF CATS AND THEIR FLEAS IN THE UNITED STATES |
| ID-31 | Michael Lappin | A FLEA AND TICK COLLAR CONTAINING 10% IMIDACLOPRID AND 4.5% FLUMETHRIN PREVENTS FLEA TRANSMISSION OF *BARTONELLA HENSELAE* IN CATS FOR 8 MONTHS |
| ID-32 | Scott Moroff | CLASSIFICATION OF *BORRELIA BURGDORFERI* INFECTION USING RESULTS OF 5 ANTIBODY TARGETS IN AN AUTOMATED SYSTEM |
| ID-33 | Kenneth Harkin | FELINE HISTOPLASMOSIS: FLUCONAZOLE THERAPY AND IDENTIFICATION OF POTENTIAL RISK FACTORS |

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**FOOD ANIMAL**

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# SMALL ANIMAL - CARDIOLOGY

**ABSTRACT C-1**

**PROGNOSTIC VALUE OF 24-HOUR AMBULATORY ECG (HOLTER) MONITORING IN BOXER DOGS.** PF Mõtsküla[1], C Linney[2], DJ Connolly[1], YM Chang[1], C Pace[1], J Dukes McEwan[2], V Luis Fuentes[1]. [1]The Royal Veterinary College, London, UK, [2]School of Veterinary Sciences, University of Liverpool, Neston, UK

Arrhythmogenic right ventricular cardiomyopathy (ARVC) in Boxers is characterized by fatty/fibrofatty infiltration of the right ventricular myocardium, often leading to ventricular ectopia, syncope and sudden cardiac death. Antemortem diagnosis depends on identification of arrhythmias on ambulatory ECG (Holter) monitoring. The prognostic value of Holter monitoring in Boxer dogs has not been clearly defined.

We aimed to identify factors associated with cardiac death. The medical records, initial Holter recordings and echocardiography results were reviewed for all boxer dogs seen at 2 centres between 2004-2011. Exclusion criteria were congenital heart disease, and a Holter recording $<19$h. Owners/veterinarians of included dogs were contacted for survival data.

Outcome data was available for 108 dogs, with 63 deaths (41 cardiac). At presentation, 83 dogs had a history of syncope, 11 had congestive heart failure (CHF) and 24 had no clinical signs. Log rank analysis showed reduced cardiac survival with presence of CHF, ventricular bigeminy/trigeminy, ventricular couplets/triplets, ventricular tachycardia (VT), and male sex, but not with a history of syncope. Univariable Cox proportional hazards analysis of Holter variables showed that mean heart rate, presence of polymorphic ventricular premature complexes (VPCs), VT, couplets or triplets, bigeminy/trigeminy, or number of VPCs/hour were positively associated with cardiac death. In a forwards stepwise multivariable model, presence of polymorphic VPCs ($p=0.001$, hazard ratio (HR) 3.2 [95% confidence interval (CI) 1.7-6.3]), presence of VT ($p=0.013$, HR 2.2 [95%CI 1.2-4.1]) and presence of SVT ($p=0.007$, HR 2.5 [95%CI 1.3-4.9]) independently predicted outcome.

Holter monitoring in Boxer dogs with suspected ARVC provides valuable prognostic information.

**ABSTRACT C-2**

**COMPARISON OF TRANSTHORACIC TWO-DIMENSIONAL ECHOCARDIOGRAPHY, THREE-DIMENSIONAL ECHOCARDIOGRAPHY, TRANSESOPHAGEAL ECHOCARDIOGRAPHY, COMPUTED TOMOGRAPHY ANGIOGRAPHY, AND GATED MAGNETIC RESONANCE IMAGING IN NORMAL CANINE HEARTS.** RC Fries[1], SG Gordon[1], MW Miller[1], AB Saunders[1], CD Hariu[1], M Bogess[1], M Lennox[2], TW Fossum[2]. [1]Texas A&M University College of Veterinary Medicine, College Station, TX, [2]The Texas A&M Institute for Preclinical Studies, College Station, TX

The purpose of this study was to determine the accuracy of two-dimensional echocardiography (2DE), three-dimensional echocardiography (3DE), transesophageal echocardiography (TEE), and computed tomography angiography (CTA) in comparison to gated cardiac magnetic resonance imaging (cMRI). Echocardiography was performed using the GE Vivid E9 with appropriate 2DE, 3DE, and adult TEE probes. CTA and cMRI images were acquired utilizing a 128-slice positron emission tomography (PET)/CT scanner and a prospective ECG-gated, 3 Tesla MRI respectively. CTA and cMRI image analysis was performed off-line using a Siemens LEONARDO 3D workstation and applicable software. Six, adult, female mixed breed dogs (18-25 kg) were anesthetized and underwent transthoracic 2DE, 3DE, and TEE (all by a single observer) CTA, and cMRI. Left ventricular internal dimension, area, and volume (in systole and diastole), stroke volume (cardiac output), and ejection fraction were assessed. Left atrial dimension, area, and volume were also evaluated.

The mean and standard deviation of the difference between cMRI and each measurement modality for the left ventricle, in diastole and systole, and left atrial volumes were estimated via maximum likelihood, with robust standard errors. For left ventricular end-diastolic volume the right parasternal four chamber view performed with 2DE (4Ch-2D) had the smallest standard deviation, while TEE and CTA had means closest to zero. For end-systolic volume the apical four chamber view performed with 3DE had the smallest standard deviation, while the mean of 4Ch-2D was closest to zero. Left atrial volume assessment with 3DE appears mildly superior to 2DE.

**ABSTRACT C-3**

**USE OF COMPUTED TOMOGRAPHY IN THE ASSESSMENT OF DOGS WITH PERICARDIAL EFFUSION.** B Bottorff, S Stieger-Vanegas, D Sisson. Oregon State University College of Veterinary Medicine, Corvallis, OR

The goal of this study was to prospectively evaluate the clinical utility of contrast-enhanced computed tomography (CT) in dogs with pericardial effusion (PE).

Eleven dogs with PE were evaluated via thoracic radiography, transthoracic echocardiography (TTE), and contrast-enhanced thoracic and abdominal CT. Two dogs had small volume PE and 9 had moderate to large volume PE with echocardiographic evidence of cardiac tamponade. TTE was performed in 9/11 dogs prior to pericardiocentesis (PCC), whereas CT was performed after PCC in 10/11 dogs. A right atrial mass was identified in 5/11 dogs via TTE and a heart base mass was identified in 1/11 dogs. One dog was diagnosed with PE due to right heart failure via TTE. Mesothelioma was later diagnosed in this dog via sternal lymph node cytology. CT detected findings consistent with TTE in all dogs with right atrial (5/5) and heart base masses (1/1). Localized pericardial hemorrhage and soft tissue changes at the site of PCC suggested a mass lesion by CT in 3/11 dogs. In all dogs mild to moderate sternal lymphadenopathy was noted using CT, which was not noted on radiographs. CT identified hepatic and splenic lesions in 5/11 and 6/11 dogs, respectively. Pulmonary metastasis was identified in 1/11 dogs via thoracic radiography and in 2/11 dogs via CT. Cardiac masses were confirmed by histopathology in 3/6 dogs.

In this population of dogs, CT and TTE had similar sensitivity for the detection of cardiac masses in dogs with PE. The value of CT resided primarily in the detection of pulmonary metastasis and extra-cardiac lesions in the liver and spleen. Pericardiocentesis performed prior to CT evaluation may confound the interpretation of CT in dogs with PE.

**ABSTRACT C-4**

**DIRECT ARTERIAL BLOOD PRESSURE MEASUREMENT USING A CAROTID CATHETER IN COMPARISON TO OSCILLOMETRIC AND DOPPLER METHOD.** K Parker, A Carr, T Duke-Novakovski. Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK

Blood pressure measurement is an important parameter when assessing hemodynamic status in animals. Cats have always been considered especially difficult to measure indirectly. This study was carried out in anesthetized cats to evaluate two newer oscillometric devices. (High Definition Oscillometry, Memodiagnostic S+B medvet, Babenhausen Germany; PC-VetGard+, DVM Solutions, San Antonio, TX), as well as ultrasonic Doppler (Parks Medical).

A total of six cats ranging from 2 to 13 years were included in this study. The anesthetized cats had the carotid artery isolated surgically and then a direct tip blood pressure transducer inserted (Millar 3.5 Fr.), then advanced approximately to the level of the aortic root. Blood pressure data were recorded continuously (Powerlab 26T, Model ML856, LabChart 7.2 software AD Instruments). The catheter was calibrated using a mercury sphygmomanometer prior to placement. The position of the cuffs of the oscillometrics was rotated between forelimbs and tail to measure blood pressure, whereas the forelimb alone was used for Doppler measurements. Blood pressure was adjusted using various isoflurane concentrations, colloids and dobutamine to

achieve hypo- (<80 mmHg), normo- (81-110 mmHg) and hypertension (>110 mmHg). Bias (indirect method minus direct) and precision (SD of the differences) were calculated

265 paired samples from the HDO and Aortic root were analyzed. Results for normotension using the HDO were Bias (-1.1) SD 5.0 mmHg for mean arterial pressure (MAP), Systolic Arterial Pressure (SAP) 3.7 (SD 10.7), and diastolic (-5.8) (SD 6.7). In hypotension (99 data pairs) bias for Mean was (-6.5) (SD 9.9), SAP 17.3 (SD 15.7) and diastolic (-0.93) (SD 14.3). In hypertension HDO underestimated SAP with a bias of (-15.0) (SD 12.8), MAP (-9.6) (SD 15.90, and diastolic (-20.9) (SD 14.3).

The VG (236 data pairs) was slightly less accurate in hypo-, normo-, and hypertensive states. In hypotension bias in MAP was 8.0 (SD 12.8 mmHg), systolic (-1.1) (SD 13.4), and diastolic (-12.3) (SD 13.4). In normotension the SAP bias was (-9.3) (SD 10.3), MAP (-12.6) (SD 7.1), and diastolic (-12.3) (SD 13.4). In hypertension the SAP bias was (-23.9) (SD 16.0), MAP (-19.3) (SD 11.3), and diastolic (-20.8)(SD 13.7).

In 66 paired samples the Doppler Technique, when using *Return to Sound* as the pressure reading on the manometer, was an accurate measurement of MAP [bias (-0.5) (SD 11.0)] but not systolic arterial pressure (SAP) [bias (-25.3) [SD 16.5].

These data indicate that HDO and VetGard appear to be accurate means of measuring blood pressure in all pressure ranges, losing some accuracy as the patient approaches hypertension. Doppler, when used for a measurement of MAP, is accurate but less so when used to assess SAP.

## ABSTRACT C-5

**CAN STRAIN RATE IMAGING DIFFERENTIATE PHYSIOLOGIC FROM PATHOLOGIC HYPERTROPHY IN THE DOG?** SM Johns, OL Nelson. Washington State University College of Veterinary Medicine, Pullman WA

The appearance of hypertrophy of the athlete's heart can be similar to hypertrophy due to heart disease or systemic hypertension. Pathologic cardiac hypertrophy can lead to decreased cardiac function and increased risk of potentially life-threatening arrhythmias. However, athletic hypertrophy is a benign process without clinical significance. The ability to more accurately differentiate between these processes will be of benefit in determining prognosis, monitoring protocols and treatment decisions.

Standard echocardiography may not reliably detect subtle changes in myocardial function. Studies in humans have shown that strain and strain rate imaging are both sensitive and specific in differentiating physiologic from pathologic (eccentric or concentric) cardiac hypertrophy. We hypothesized that strain and strain rate imaging will differentiate physiologic from pathologic hypertrophy in dogs.

We performed echocardiograms on 10 Silken Windhounds prior to initiation of athletic training. Athletic training of 9 of the dogs was instituted in the form of coursing for a gradually increasing number of miles per week. Echocardiograms were repeated 9 weeks later to evaluate changes in left ventricular diameters, wall thickness, mitral inflow, tissue Doppler, strain, and strain rate. In addition, 5 dogs with varying degrees of pathologic concentric left ventricular hypertrophy due to cardiac disease were also evaluated for comparison. Preliminary results indicate no change in left ventricular diameter, wall thickness, mitral inflow pattern, tissue Doppler pattern, strain, or strain rate in Silken Windhounds before and after 9 weeks of athletic training; a longer period of training may be needed to detect differences. There was no significant difference between dogs with pathologic hypertrophy and conditioned windhounds for standard echocardiography parameters of ejection fraction, fractional shortening, mitral inflow E/A, mitral E deceleration time, or tissue Doppler E' or A'. When compared to conditioned windhounds, dogs with pathologic hypertrophy had significantly increased mitral inflow E/E' ratios (12.2 vs. 5.9, $p = 0.018$), significantly decreased systolic longitudinal velocity (2.218 m/s vs. 4.718 m/s, $p < 0.01$) and E wave longitudinal velocity (-2.034m/s vs. -4.27m/s, $p = 0.028$), and significantly increased A wave longitudinal strain rate (1 vs 0.41, $p = 0.039$). These primarily diastolic strain imaging indices may be useful to differentiate pathologic concentric hypertrophy from changes that may be associated with athletic training.

## ABSTRACT C-6

**OPTIMIZATION OF IMPLANTABLE CARDIOVERTER DEFIBRILLATOR (ICD) DEFIBRILLATION THRESHOLDS IN DOGS.** C Saelinger, J Vila, P Queiroz-Williams, R Pariaut. Dept. of Veterinary Clinical Sciences, Louisiana State University, Baton Rouge, LA

In dogs, treatment of malignant ventricular arrhythmias is centered on the administration of antiarrhythmics. Despite a reduction of clinical signs, the risk for sudden death is not decreased. In human patients, utilization of implantable cardioverter defibrillators (ICDs) in conjunction with antiarrhythmics is standard of care to reduce clinical signs and sudden death from ventricular arrhythmias. As ICDs become more available for implantation in dogs, optimization of placement and programming while considering the unique canine anatomy and physiology is crucial.

The objectives of this study were to: 1) implant ICDs in healthy canines in an anatomical location that allows successful, low energy defibrillation (at least 10 J safety margin) and 2) identify the shock configuration that achieves the lowest defibrillation thresholds (DFTs), i.e. the lowest energy that terminates ventricular fibrillation (VF). We hypothesized that a fixed pulse duration shock configuration, as determined by a surrogate of the cardiac tissue time constant, results in a lower DFT compared to a fixed tilt shock configuration.

Ten healthy mongrels (24.6 kg ± 3.86 kg) were included in this study. Under fluoroscopic guidance, a 65-cm, 2-coiled endocardial lead was placed into the right ventricular apex through the left jugular vein. The proximal portion of the lead was tunneled subcutaneously under the left shoulder and secured into the generator in a subcutaneous pocket between the $4^{th}$ and $6^{th}$ left intercostal spaces, above the left ventricle. Capture testing was performed. VF was induced via the shock-on-T method and the upper limit of vulnerability theory was used to reduce the number of VF inductions needed to determine the DFT. DFT testing was performed with both fixed pulse and fixed tilt shock configurations in random order. Plasma Troponin I (TnI) levels were measured before induction of VF and after the first and second DFT protocols. Paired t-test was used to analyze DFT results and repeated measures ANOVA was used for TnI levels. All values are shown as mean ± SD. $P \leq 0.05$ was considered statistically significant.

One subject died during the induction of anesthesia. Nine remaining dogs underwent successful ICD implantation and DFT testing. Overall mean DFT was 9.19 ± 3.88 J. DFTs for fixed pulse (9.06 ± 4.195 J) and fixed tilt (9.33 ± 3.783 J) shock configurations were not significantly different. There was a significant difference in the TnI levels between baseline (0.034 ± 0.028 ng/mL) and following determination of the first (0.124 ± 0.055 ng/mL, $P \leq 0.05$) and second (0.217 ± 0.104 ng/mL, $P \leq 0.0001$) DFT values.

Important results of this study are: 1) The proposed ICD placement consistently resulted in a very low and more than adequate (more than 20 J below maximum energy of the device) DFT in normal canines, 2) there was no difference between the fixed pulse and fixed tilt shock configurations, and 3) the increase in TnI was not considered to be clinically significant.

## ABSTRACT C-7

**ATRIAL FIBRILLATION IS A NEGATIVE PROGNOSTIC INDICATOR IN LARGE BREED DOGS WITH MYXOMATOUS MITRAL VALVE DEGENERATION AND CONGESTIVE HEART FAILURE.** SW Jung, LG Griffiths, MD Kittleson, WR Pritchard. Veterinary Medical Teaching Hospital, University of California-Davis, Davis, CA

The outcome of congestive heart failure (CHF) due to myxomatous mitral valve degeneration (MMVD) in small breed dogs has been well documented. MMVD also occurs in large breed dogs (> 15 kg) but the disease characteristics in this cohort are poorly documented and are somewhat different from small breed dogs. For example, large breed dogs with MMVD usually have overt evidence of myocardial dysfunction when CHF develops and appear to develop atrial fibrillation (AF) more frequently than small dogs. The aim of the present study was to evaluate the prevalence and prognostic significance of AF in large breed dogs with MMVD and CHF.

Medical records between January 2005 and December 2010 were reviewed retrospectively and 63 large breed dogs with MMVD and

CHF that reached the primary endpoint (cardiac-related death) were identified. CHF was defined as pulmonary edema, ascites or both due to severe cardiac disease. The onset of CHF was defined as the time when diuretic drug administration was initiated. The median age and body weight were 11 years of age (range: 6-15 years) and 24 kg (range: 16-64 kg), respectively. The median ratio of left atrial diameter to aortic root diameter on 2D short-axis view was 2.5 (range: 1.7-3.7). The median shortening fraction was 36% (range: 22-55%). The median survival time of all dogs from the identification of CHF by the referring veterinarian to cardiac death was 172 days (range: 9-879 days). Thirty three dogs (52%) had sustained atrial fibrillation. Their median heart rate before and after pharmacological rate control was 220 beats/min (range: 160-270 beats/min) and 177 beats/min (range: 84-240 beats/min), respectively. Kaplan-Meier statistics revealed that dogs in atrial fibrillation had a significantly shorter median survival time (142 days; range: 9-478 days) compared with dogs in sinus rhythm (234 days; range: 13-879 days) ($P = 0.006$).

We conclude that the prevalence of AF in large breed dogs in CHF due to MMVD is high and that AF adversely impacts survival time. A prospective clinical trial is warranted to confirm or refute the findings from this study.

## ABSTRACT C-8

**MALIGNANT VENTRICULAR ARRHYTHMIAS ARE ASSOCIATED WITH DILATED CARDIOMYOPATHY IN GREAT DANES.** H Stephenson[1], S Fonfara[1], J López-Alvarez[1,2], J Dukes-McEwan[1]. [1]University of Liverpool Small Animal Teaching Hospital, Liverpool, UK, [2]The Royal Veterinary College, Hertfordshire, UK

The Great Dane (GD) is commonly affected by dilated cardiomyopathy (DCM) and has the shortest median survival time. Unlike some breeds, ventricular arrhythmias (VA) and sudden death (SD) are infrequently reported in GD, however recent prospective screening in the UK identified a high prevalence of VA in GDs. Furthermore SD is frequently reported by owners and breeders. This study aimed to investigate the prevalence, significance and association of VA and DCM in GD.

As part of an ongoing longitudinal study, asymptomatic dogs were screened by physical examination, blood samples, Doppler echocardiography (ECHO) and ECG. Pedigree and historical data were collected. 24 hour ECG (Holter) monitors were fitted to selected GDs. Dogs were monitored by telephone follow-up, repeat screening and post-mortem (PM) if possible.

Over three years, 111 dogs were screened. 3 were excluded due to other disease. 4 dogs had no ECHO or ECG performed. VA were identified on ECG in 29.8% of dogs (31/104). Holter monitors were fitted in 24 dogs and 54% (13/24) had VA. 7/38 dogs with VA on either ECG or Holter died suddenly. 19 dogs belonged to one family with a high prevalence of SD. Both VA (10/19) and preclinical DCM (5/19) were identified in these dogs. PM in 2 dogs showed myocardial fibrofatty infiltrate.

In conclusion, our data shows that VA occur commonly in GD in the UK. Both VA and DCM occur within the same family. The high prevalence of VA suggests that this may be an important cause of SD in GDs.

## ABSTRACT C-9

**UTILZATION OF TRANSESOPHAGEAL ATRIAL PACING TO OBTAIN SINUS NODE RECOVERY TIME IN DOGS.** IZ Giatis[1], HW Green III[1], E Chatel[2], RA Sanders[2]. [1]Purdue University College of Veterinary Medicine, West Lafayette, IN, [2]Michigan State University, College of Veterinary Medicine.

Sick sinus syndrome (SSS) is classified into two etiologic categories: extrinsic and intrinsic. Extrinsic SSS, a vagally influenced bradycardia must be differentiated from intrinsic SSS, true sinoatrial node dysfunction. The atropine response test (ART) has been utilized to rule out extrinsic causes of SSS; however, there are variable responses with intrinsic SSS to the ART. Thus sinus node recovery time (SNRT) via intracardiac electrophysiology studies is often utilized as a more reliable method to measure sinus node function in humans. Similar methodology is particularly limited in veterinary medicine due to the invasive nature of this test and the extremely limited availability of expensive electrophysiological equipment to veterinary cardiologists. Thus diagnosis of dogs with intrinsic SSS has been restricted in veterinary medicine to ART. Transesophageal atrial pacing (TEAP) is an easily obtainable and cost-effective means to temporarily pace the atrium in dogs. We sought to compare SNRT results obtained by using TEAP to those obtained by standard intracardiac atrial pacing (ICAP).

Seven juvenile beagle dogs underwent general anesthesia followed by oral placement of a multipolar electrophysiology catheter into the esophagus to the level of the carina. Jugular venous access was obtained via modified Seldinger technique utilizing a 6 Fr introducer system following which a steerable multipolar electrode was fluoroscopically guided to the level of the high right atrium. The SNRT was obtained following atrial pacing for 30 seconds at 500ms, 400 ms, 300 ms, and 200ms cycle lengths respectively with each technique. Dogs were randomly selected to receive TEAP or ICAP first. Corrected sinus node recovery times (cSNRT) were also calculated.

Three male and four female dogs (10.9 ± 1.26 kgs) were evaluated in this study. No statistical differences were noted in mean SNRT and cSNRT values respectively with either technique (See table 1). Data is demonstrated as the mean ± standard deviation in milliseconds where applicable.

We conclude that TEAP pacing demonstrates similar SNRT times compared to those utilizing standard intracardiac technique normal dogs. As such TEAP may be used when standard studies are not available. Further assessment utilizing dogs with sinus node disease need to be validated.

## ABSTRACT C-10

**LONG-TERM EVALUATION OF CARDIAC SIZE AND FUNCTION IN 71 DOGS FOLLOWING PATENT DUCTUS ARTERIOSUS CLOSURE.** AB Saunders, SG Gordon, CD Hariu, RC Fries, JA Carlson, RL Winter, MW Miller. The Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX

Herein we report cardiac size and function at baseline (B) and >12 months follow-up (F) in a cohort of dogs with PDA. Between July 1996-November 2009, 523 dogs presented to TAMU for PDA. Baseline data including signalment, history, physical examination, diagnostic imaging, type of closure were obtained from medical records. Follow-up was available on 71 dogs.

All had characteristic murmurs of PDA; 55 were female. The most common breed was Chihuahua (10). PDA closure methods included ligation (28), coil (20), Amplatz® canine duct occluder (18), Amplatzer® vascular plug (5). Concurrent congenital heart disease was identified in 7. Chronic valve disease was present in 1 (B) and 16 (F). Residual ductal flow was documented within 24-hours in 9 and in 4 at F. Median time from closure to F was 48.9 months (range, 13.0-125.3). Results are reported as mean±SD (range) at B and F. Age (months) was 16.6±21.2 (2.2-97.9) and 69.6±29.9 (21.3-151.9). Weight (kg) was 8.1±7.9 (0.3-37.2) and 13.4±10.6 (1.3-36.5). Vertebral heart size was 11.7±1.1 (9.2-14.1) and 10.5±0.6 (9.0-12.6). LVIDdN was 2.21±0.54 (1.31-4.28) and 1.54±0.25 (1.10-2.20). LVIDsN was 1.37±0.41 (0.60-2.60) and 1.03±0.26 (0.50-2.90). FS% was 36.6±8.2 (13.0-54.0) and 29.7±10.3 (9.0-56.0). LA/Ao mmode was 1.43±0.28 (0.84-2.02) and 1.21±0.15 (0.87-1.50). P-value was <0.0001 for all variables compared. VHS was >10.5 in 56/66 (B) and 18/70 (F). The

| Cycle Length | TEAP SNRT(ms) | ICAP SNRT(ms) | P value | TEAP cSNRT(ms) | ICAP cSNRT(ms) | P value |
|---|---|---|---|---|---|---|
| 500 ms | 798.5 ± 103.7 | 744.3 ± 208.9 | .608 | 149.5 ± 103 | 132.8 ± 103.7 | .785 |
| 400 ms | 824.3 ± 210.6 | 827.3 ± 202.8 | .981 | 199.9 ± 124.8 | 208.3 ± 141.0 | .915 |
| 300 ms | 873.17 ± 261.8 | 889.8 ± 280.6 | .918 | 246.5 ± 161.7 | 265.8 ± 198.3 | .858 |
| 200 ms | 1268.4±921.3 | 1307.8±1000.2 | .860 | 680.7±855.5 | 718.3±958.3 | .886 |

number of dogs >95% CI for LVIDdN and LVIDsN was 53/66 (B), 36/66 (B) and 6/70 (F), 11/70 (F) respectively suggesting normalization of cardiac size ranging from 69.4-88.7% at long-term follow-up based on these values.

## ABSTRACT C-11

## CANINE CARDIAC TROPONIN I SIGNIFICANTLY COMPLEMENTS ESTABLISHED PROGNOSTIC COMPOSITE SCORE IN DOGS WITH SYSTEMIC INFLAMMATION.

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Composite scoring models based on clinical and paraclinical observations are used in intensive care units (ICUs) for stratification of disease severity and early assessment of prognosis for survival. Main components of these models are markers of organ dysfunction. However, the significance of myocardial injury was not included when the models, e.g. the Acute Patient Physiologic and Laboratory Evaluation (APPLE) score in dogs, were developed. In non-cardiac critical disease, myocardial injury as detected by cardiac troponin I (cTnI) has been linked to high ICU mortality in several human studies. The purpose of this study was to examine the prognostic value of cTnI in critically ill dogs with systemic inflammation as an addition to the APPLE scoring system.

Dogs admitted to the ICU (University of Pennsylvania) were prospectively included over a 3 month period. Echocardiography was performed on all dogs, and dogs with evidence of primary structural cardiac disease were excluded. Systemic inflammation at ICU admission was defined as a serum concentration of C-reactive protein (CRP) > 35 mg/L within the first 24 hours in a dog with clinical evidence of inflammation. Samples for cTnI analysis were obtained at ICU admission and for CRP analysis at admission and 12-24 hours later. The APPLE score was calculated for each dog within the first 24 hours of ICU admission. Outcome (death versus survival) was determined 28 days after admission. Areas under receiver operator characteristic curves (AUC-ROC) were used to evaluate the performance of cTnI and the APPLE score as prognostic markers of outcome. cTnI concentrations were logarithmically transformed to optimize distribution for multiple logistic regression analysis (MLR), and MLR was performed to evaluate whether cTnI made a significant contribution to the APPLE score (Likelihood ratio test). Level of significance was set to $P < 0.05$.

Forty-two dogs were included, with a mortality rate of 26.2% (n = 11). cTnI concentrations were (median [range]) 0.416 [0.004-141.5] ng/mL. 88.1% (n = 37), including all non-survivors, had cTnI concentrations above reference limit (0.07 ng/mL). AUC-ROC [95 % CI] for cTnI and the APPLE score as predictors of outcome were 0.801 [0.649;0.907] and 0.776 [0.621;0.889], respectively, thus both were individually significant prognosticators. Graphing the cTnI concentration against the APPLE score suggested that cTnI complemented the APPLE score in providing additional prognostic specificity, improving the positive predictive value of the model. This was confirmed by the MLR, where cTnI significantly contributed in a model combining the APPLE score and cTnI (coefficient estimate: 0.44 [0.02;0.86], corresponding to an odds ratio for 28-day mortality of 1.6 for each 10-fold increase in cTnI).

We concluded that the inclusion of canine cTnI as a marker of myocardial injury in critically ill dogs with systemic inflammation significantly complemented the established prognostic composite score APPLE, apparently by improving prognostic specificity.

## ABSTRACT C-12

## CLINICAL EVALUATION OF THE 3M™ LITTMANN® MODEL 3200 ELECTRONIC STETHOSCOPE IN CATS.

KA Blass, KE Schober, BA Scansen, LC Visser, J Lu, D Smith, J Ward, JD Bonagura. The Ohio State University, Columbus, OH

Cardiac auscultation is of utmost importance in the physical examination of cats. Determination of heart rate and identification of murmurs, gallops, and clicks may help in the identification of cats with heart disease. However, auscultatory findings may be dynamic, temporary, affected by ambient noise, and influenced by observer training and expertise, thus being subject to relevant observer variation. The objective of this study was to evaluate a fully electronic stethoscope in cats.

Consecutive cats undergoing echocardiography were enrolled. Cats were ausculted with a traditional stethoscope (WA Tycos Harvey™ Elite®) by 2 observers and heart sounds were recorded using an electronic stethoscope (3M™ Littmann® stethoscope Model 3200) for off-line analysis. Results were compared using standard statistical analysis. In addition, digital recordings were assessed by 8 independent observers with various levels of expertise and compared using interclass correlation (IC) and Cohen's weighted kappa analyses.

Of a total of 150 cats, 93 (62%) had cardiac murmurs, 33 (22%) systolic clicks, 20 (13%) gallop sounds, and 10 (7%) cardiac arrhythmias. There was good total agreement (86% to 95%) between the 2 observers using the Tycos. There was significant agreement ($P < 0.001$) between results from the Tycos and the 3M™ Littman® stethoscope. The Littman was more sensitive compared to the Tycos with regard to gallop sounds and clicks. IC among observers was good to excellent (ICC 0.75 to 0.96).

The 3MTM Littmann® stethoscope is useful for detecting abnormal heart sounds and murmurs with high sensitivity and specificity and can be recommended for use in feline practice.

## ABSTRACT C-13

## ANALYSIS OF ATRIAL REPOLARIZATION WAVE ($T_a$) IN DOGS WITH THIRD DEGREE ATRIOVENTRICULAR BLOCK.

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Data on atrial repolarization in dogs are scarce since the QRS complex deflections usually obscure $T_a$ wave. Electrocardiographic features of $T_a$ wave can be better analyzed in patients with third degree atrioventricular (AV) block since, in this condition, the presence of AV dissociation makes the $T_a$ wave and the QRS complex uncoupled. Atrial repolarization wave varies according to the autonomic status and the presence of atrial injuries. The aim of the study was to describe the electrocardiographic characteristics of $T_a$ wave in dogs with third degree AV block, regular atrial cycle length and normal cardiac chamber size. Clinical records of 52 dogs with third degree AV block were retrospectively analyzed. Ten dogs were excluded because of the presence of ventriculophasic sinus arrhythmia and 6 dogs because of the presence of atrial enlargement. The sample group included 21 males and 15 females with a mean age of 110.9 (confidence interval 95% [CI 95%] 99.5-122.4) months and mean weigh of 29.0 kg (CI 95% 23.5-34.4). Standard 12-lead electrocardiograms were acquired with a digital system for a subsequent off-line processing. Each parameter was evaluated by a single operator with three repeated measurements. Normalcy was assessed by Shapiro Wilks test, correlation was calculated by Pearson, Sperman and Kendall tests, on variable that showed a Pearson correlation a linear regression was performed. A multivariate analysis was conducted by generalized linear equation. Repeatability was tested by intra-class coefficient of correlation using Shrout-Fleiss reliability test. $T_a$ wave was detectable in 80.6 % of the cases in inferior leads (II,III and aVF), in 22.2 % in lead I, in 44.4% in lead aVL, in 75 % in lead aVR and in 67.6 % in precordial leads. $T_a$ wave showed a negative polarity in lead I, II, III and aVF and a positive polarity in lead aVL and aVR with a mean electrical axis in the frontal plane of -114.3° (CI 95% -121.6 - -107.0). In all precordial leads $T_a$ wave showed a negative polarity. The mean duration and the mean amplitude of the $T_a$ wave in lead II were, respectively, 140.2 msec (CI 95% 127.5-152.9) and -0.09 mV (CI 95% -0.1 - - 0.08) with a $T_a$/P duration ratio of 2.3 and a $T_a$/P amplitude ratio of -0.35. A linear correlation between $T_a$ wave duration and P-$T_a$ interval duration was found. Multivariate analysis showed significant correlations between $T_a$ wave amplitude and examined lead, $T_a$ wave duration and weight, P-$T_a$ interval duration and atrial rate, $T_a$ wave amplitude and duration with weight and $T_a$ wave duration and P-$T_a$ interval duration with weight. $T_a$ wave measurements were repeatable ($T_a$ voltage K 0.759; $T_a$ duration K 0.942). In agreement with previous studies, we found opposite polarity of the P and the $T_a$ waves regardless of which lead was analyzed, and a positive correlation between atrial

rate and P-$T_a$ interval duration. In contrast with previous veterinary study, we found no correlation between P wave and $T_a$ wave duration. This study provides a full description of electrocardiographic features of the atrial repolarization phase in dogs with third degree AV block with possible extension of the results to the normal population. Further studies are needed to analyze the atrial repolarization phase in relation to the atrial arrhythmic propensity, as does QT interval analysis in the case of ventricular arrhythmias.

**ABSTRACT C-14**

**STRUCTURAL AND MOLECULAR PATHOLOGY OF THE ATRIUM IN BOXER ARRHYTHMOGENIC CARDIOMYOPATHY.** J Vila[1], EM Oxford[2], C Saelinger[1], NS Moise[2], PR Fox[3], R Pariaut[1]. [1]Dept. of Veterinary Clinical Sciences, Louisiana State University, Baton Rouge, LA, [2]College of Veterinary Medicine, Cornell University, Department of Clinical Sciences, Ithaca, NY, [3]The Animal Medical Center, 510 E 62nd St, New York, NY

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is an inherited myocardial disease characterized by fatty or fibrofatty infiltration of the myocardium. While the specific mechanism is still unknown, ARVC is recognized as a disease affecting the cardiac intercalated disc and more specifically the desmosomal proteins that compose it. ARVC is classically associated with ventricular arrhythmias as it primarily affects the ventricular myocardium, although atrial arrhythmias and atrial histopathological changes characteristic of ARVC have occasionally been reported in both Boxers and humans. However, the extent of atrial involvement in ARVC has not been investigated.

The objective of the study was to apply histopathology, immunochemical detection, immunolocalization and transmission electron microcopy (TEM) techniques to better characterize the distribution of desmosomal and gap junction proteins at the intercalated disc level in the atria of Boxers with ARVC. We hypothesized that histological changes consistent with ARVC and alterations of the proteins of the intercalated disc are present in the atria of Boxer dogs with ARVC and may represent the anatomic substrate for atrial arrhythmias.

The hearts from 14 healthy mongrel dogs and 14 Boxer dogs with confirmed ARVC were studied. Right and left atrial sections from 10 Boxers were examined by immunofluorescent microscopy. Frozen tissue samples from 8 Boxers were used for Western blot (WB) analysis. The intercalated disc proteins investigated were Connexin 43 (Cx43), Connexin 45, Connexin 40, Plakoglobin, Plakophilin 2, Desmoplakin, and Cadherin. Electron microscopy was performed on the right and left atrial sections of 2 Boxers and 2 controls. Western blot band relative density was calculated for all the protein tested using the NIH software ImageJ. Student-t test was used to analyze the WB results. $P \leq 0.05$ was considered statistically significant.

Western blots indicated a significant decrease of Cx43 in the right atrium of ARVC affected Boxers (relative density Cx43/Actin 0.68 +/- 0.23 SD) compared to controls (relative density Cx43/Actin 1.115 +/- 0.3 SD). There was no difference between controls and Boxers for the other proteins investigated. Immunofluorescence analysis showed that the concentration of Cx43 was decreased in the left and right atrium of ARVC affected Boxers compared to controls. However, the intensity of the Cx43 appeared to be the same in the two groups. TEM showed disruption of the intercalated disc in Boxers.

Our data suggests that the atrial myocardium is also affected during ARVC. The decrease in Connexin 43 in conjunction with the fatty or fibro-fatty infiltration of the myocardium could represent the substrate for the atrial arrhythmias that can be seen with ARVC.

**ABSTRACT C-15**

**INCIDENCE OF HEART DISEASE IN CATS WITH ELECTROCARDIOGRAPHIC EVIDENCE OF LEFTWARD MEAN ELECTRICAL AXIS SHIFT.** J Vitt, MC Machen, MA Oyama. University of Pennsylvania, Philadelphia, PA

Detection of feline heart disease is difficult as physical examination, radiographic, and electrocardiographic findings can be relatively non-specific. Previously, leftward mean electrical axis shift (LAS), has been proposed as a risk factor for feline heart disease. We sought to determine if presence of LAS was predictive for echocardiographic heart disease in a retrospectively-identified population of cats referred to a teaching hospital for cardiac evaluation.

The mean electrical axis (MEA) was calculated for 153 cats. Cats were classified as normal (N), or equivocal (E), or having isolated dynamic right ventricular outflow tract obstruction (DRVOTO), or heart disease (HD) based on echocardiography.

The results were as follows: LAS (-1° to -89°) n = 28 (N, 2; HD, 18; DRVOTO, 1; E, 7); 64% LAS had HD. N MEA (0°-160°) n = 102 (N, 21; HD, 54; DRVOTO, 7; E, 20); 53% N MEA had HD. Right MEA (161° to -91°) n = 18 (N, 2; HD, 13; DRVOTO, 0; E, 3). Indeterminant MEA (-90°) n = 18 (N, 0; HD, 5; DRVOTO, 0; E/R, 0). Chi-square analysis revealed that the proportion of HD vs. N in cats with N MEA vs. LAS was not statistically different ($P = 0.14$). No significant difference was found regardless of whether E were analyzed as N or HD.

In a referral population of cats, the presence of LAS was not predictive of HD on echo compared to cats with a normal MEA, indicating that LAS was not a risk factor for presence of HD in the population. Further studies are needed to identify other parameters.

**ABSTRACT C-16**

**NT-PROBNP AS A SCREENING TOOL TO DETECT CLINICALLY SIGNIFICANT HEART DISEASE IN ASYMPTOMATIC CATS WITH HEART MURMURS AND/OR GALLOP RHYTHMS.** JM Mulz, DP Schrope. Oradell Animal Hospital, Paramus, NJ

The purpose of this study was to determine whether NT-proBNP could be used as a screening tool to detect clinically significant disease in patients with an indication of underlying cardiac disease based on physical exam abnormalities. Clinically significant disease was defined as structural cardiac disease severe enough to warrant follow-up within four months or recommend medications.

A total of 86 cats were enrolled in the study. The cats either presented for a routine wellness examination to the Oradell Animal Hospital, or were examined at a local animal shelter. Each cat identified had an auscultable murmur or gallop rhythm detected by a general practitioner and was otherwise asymptomatic. A complete blood count, serum chemistry, T4 level, NT-proBNP level and Doppler blood pressure were performed. Of the 86 cats, 9 cats were excluded based on bloodwork or blood pressure abnormalities and six cats were lost to follow-up, leaving a total of 71 cats in the study population. The 71 cats had echocardiograms performed by a single, experienced investigator (DPS) who performed echocardiograms for several studies concurrently, blinding the investigator as to whether or not the cat had a murmur. Cats were classified as normal (17) or having equivocal (13), mild (24), moderate (12) or severe disease (5) based on the echocardiographic findings. Due to the low numbers of cats with moderate and severe disease, these groups were combined, as all cats in these groups were considered to have clinically significant disease.

When compared to the echocardiographic results, NT-proBNP reliably differentiated mild disease from normal ($p = 0.0315$) and equivocal disease ($p = 0.0410$), as well as differentiating moderate and severe disease from normal ($p = 0.0071$), equivocal ($p = 0.0073$) and mild disease ($p = 0.0157$). NT-proBNP did not differentiate between normal and equivocal disease ($p = 0.682$). This study suggests that NT-proBNP may be a reliable screening tool to detect clinically significant disease in asymptomatic cats with auscultatory abnormalities.

**ABSTRACT C-17**

**HEART RATE AND ARRHYTHMIA FREQUENCY OF NORMAL CATS COMPARED TO CATS WITH HYPERTROPHIC CARDIOMYOPATHY.** B Smouther, L Lehmkuhl, D Adin, T Nguyenba. MedVet Medical and Cancer Centers for Pets, Worthington, OH

Hypertrophic cardiomyopathy (HCM) often has a long asymptomatic period, but can result in congestive heart failure,

thromboembolism, or sudden cardiac death. Although sudden death is often attributed to a fatal arrhythmia, there is little information in the literature regarding arrhythmia frequency in this population of cats. The purpose of this study was to describe the heart rate, arrhythmia frequency, and arrhythmia severity in a population of asymptomatic HCM cats compared to a normal population. Additionally, we assessed the ability of in-clinic measures of heart rate and rhythm (auscultation (AUSC) and 2-minute electrocardiogram (ECG)) to predict average heart rate (24AVE) and arrhythmia frequency at home assessed by 24 hour Holter monitor.

Seventeen cats with HCM and 15 normal cats were prospectively evaluated by AUSC, echocardiogram, blood pressure, ECG, and 24 hour Holter monitor.

All (17/17) HCM cats had ventricular arrhythmias (geometric mean, 124 complexes) with 82% (14/17) exhibiting complex arrhythmias (couplets, triplets, or ventricular tachycardia). Most (14/15) normal cats had ventricular arrhythmias (geometric mean, 4 complexes), but only 20% (3/15) exhibited complexity. HCM cats had significantly more total ventricular complexes (TOT), ventricular premature complexes (VPCs), and accelerated idioventricular rhythm than normal cats ($p < 0.0001$, $p < 0.0001$, and $p = 0.01$, respectively). There was no significant correlation between left ventricular outflow tract velocity and frequency of TOT or VPCs ($p = 0.6$, $p = 0.4$ respectively). Eighty eight percent (15/17) of HCM cats had supraventricular arrhythmias (geometric mean, 9 complexes) with 23% (4/17) exhibiting complexity. Sixty percent (9/15) of normal cats had supraventricular arrhythmias (geometric mean 1 beat) with 13% (2/15) exhibiting complexity. HCM cats had significantly more supraventricular complexes than normal cats ($p = 0.0148$). Additionally, 100% of HCM cats (17/17) had arrhythmias on Holter monitor, while only 18% (3/17) had arrhythmias on ECG. All normal cats (15/15) also had arrhythmias on Holter monitor, and none had arrhythmias on ECG.

There was no difference in heart rate between HCM cats and normal cats regardless of modality used (AUSC $p = 0.8$, ECG $p = 0.3$, Holter 10% $p = 0.99$, Ave $p = 0.97$, 90% $p = 0.2$. When comparing 24AVE and ECG, bias was 30 bpm with limits of agreement of +/- 49 bpm. When comparing 24AVE to AUSC, bias was 21bpm with limits of agreement +/- 54 bpm.

HCM cats have more frequent and complex ventricular and supraventricular arrhythmias than normal cats. Agreement between 24 hr Holter and in-clinic AUSC or ECG for HR assessment and arrhythmia detection was poor. Long term studies are needed to determine if this increased arrhythmia frequency and severity is related to sudden cardiac death or survival.

## ABSTRACT C-18

### EFFECT OF ATENOLOL ON HEART RATE, ARRHYTHMIAS, BLOOD PRESSURE, AND DYNAMIC LEFT VENTRICULAR OUTFLOW TRACT OBSTRUCTION IN CATS WITH ASYMPTOMATIC HYPERTROPHIC CARDIOMYOPATHY.

B Smouther[1], D Adin[1], L Lehmkuhl[1], T Nguyenba[1], K Meurs[2], B Maran[3]. [1]MedVet Medical and Cancer Centers for Pets, Worthington, OH, [2]North Carolina State University College of Veterinary Medicine, Raleigh, NC, [3]Washington State University College of Veterinary Medicine, Pullman, WA

Atenolol is commonly prescribed to asymptomatic cats with hypertrophic cardiomyopathy (HCM) for theoretical benefits including heart rate (HR) reduction, increased ventricular filling time, decreased myocardial oxygen demand, arrhythmia reduction, and dynamic left ventricular outflow tract (LVOT) obstruction reduction. The purpose of this study was to describe the HR, murmur grade, arrhythmias, blood pressure (BP), and LVOT velocity in cats with HCM pre (PRE) and post (POST) atenolol administration.

Seventeen cats with HCM were prospectively evaluated by auscultation (AUSC), echocardiogram, BP, 2-minute electrocardiogram (ECG), and 24 hour Holter monitor. Cats were re-evaluated after 2-4 weeks of atenolol administration. All cats were negative for known beta-1 adrenergic receptor polymorphisms.

The HR decreased after atenolol for all HR modalities measured: AUSC from 191 bpm to 153 bpm ($p < 0.0001$), ECG from 206 bpm to 154 bpm ($P < 0.0001$), and Holter 90%, average, and 10% HR from 245 bpm to 196 bpm ($p < 0.0001$), 170 bpm to 135 bpm ($p < 0.01$), and 133 bpm to 104 bpm ($p < 0.001$) respectively.

There was a decrease in total ventricular ectopic complexes (TOT) and ventricular premature complexes (VPCs) after atenolol. TOT decreased from a geometric mean of 123 to 22 per 24 hr (82% reduction, $p = 0.004$). VPCs decreased from a geometric mean 61 to 15 per 24hr (75% reduction, $p < 0.0001$). For TOT, 5 cats had > 85% reduction, 5 cats had 50-85% reduction, and 7 cats had < 50% reduction. For VPCs, 4 cats had > 85% reduction, 10 cats had 50-85% reduction, and 3 cats had < 50% reduction. There was no decrease in accelerated idioventricular rhythm (geometric mean 6 to 3 per 24hr, $p = 0.6$) or supraventricular premature complexes (geometric mean 4 to 2 per 24 hr, $p = 0.13$).

Murmur grade decreased post atenolol ($p < 0.0001$), as did LVOT velocity (mean 3.3m/s to 1.5 m/s, $p < 0.0001$). There were 11 HCM cats with LVOT obstruction secondary to systolic anterior motion of the anterior mitral valve leaflet (SAM). Seven cats had complete resolution of obstruction and SAM, 3 cats had > 50% reduction in LVOT velocity, and 1 cat had no decrease in LVOT velocity or resolution of SAM. BP did not change post atenolol ($P = 0.2$).

Atenolol decreases HR, murmur grade, ventricular arrhythmias, and LVOT obstruction in cats with HCM without lowering BP. Further studies are needed to determine if long-term outcome is affected by atenolol administration.

## ABSTRACT C-19

### THE EFFECT OF ENALAPRIL ON FUROSEMIDE-ACTIVATED RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS) IN NORMAL DOGS.

AC Lantis[1], CE Atkins[2], M Ames[2]. [1]Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, [2]North Carolina State University College of Veterinary Medicine, Raleigh NC

The purpose of this study was to evaluate the ability of the ACE-inhibitor, enalapril, to suppress circulating RAAS activation in normal dogs treated with furosemide. We hypothesized that enalapril would suppress plasma ACE activity and furosemide-induced RAAS activation, as evaluated by urinary aldosterone:creatinine ratio.

Sixteen healthy hound dogs were used. Group1 (control) received furosemide (2 mg/kg PO, BID) for 7 days. Group 2 received furosemide (2 mg/kg PO, BID) and enalapril (0.5 mg/kg every 12 hours) for 7 days. To measure the response of the RAAS to the medications, plasma ACE activity and urinary aldosterone:creatinine ratio (A:Cr) were obtained on days -1, -2, 1, 4 and 7.

Urinary aldosterone secretion, as indicated by the A:Cr, increased nearly 3-fold by furosemide monotherapy. This was maintained for the 7 days of the study. The addition of enalapril to group 2 dogs successfully suppressed plasma ACE activity but did not significantly reduce urinary aldosterone excretion.

We have shown that, while enalapril successfully blunted ACE activity, it did not reduce urinary aldosterone excretion. The absence of circulating RAAS suppression accompanied by ACE activity inhibition defines aldosterone breakthrough. Studies in clinical patients are warranted to determine if and when aldosterone breakthough occurs in dogs receiving ACE-I for cardiac disease, glomerular disease, or hypertension.

## ABSTRACT C-20

### THE EFFECT OF HIGH DOSE PIMOBENDAN ON THE FUROSEMIDE-INDUCED RENIN-ANGIOTENSIN-ALDOSTERONE-SYSTEM (RAAS).

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Pimobendan confers positive inotropic and vasodilatory effects, the latter known to activate the RAAS, which is harmful in heart failure. Evaluation of 118 dogs (from 4/2007- 8/2011)

receiving pimobendan ≥TID for refractory heart failure revealed 30.5% to be receiving ≥1.2mg/kg/day (mean 2.20mg/kg/day; range 1.22-3.90mg/kg/day; recommended dosage 0.5-0.6mg/kg/day), indicating the potential negative clinical impact of a stimulatory RAAS effect of 'high-dose' pimobendan.

The purpose of this study was to determine if high-dose pimobendan (0.6mg/kg PO q12h) potentiates furosemide-induced RAAS activation. We hypothesized, based on a previous study, that high-dose pimobendan, when used concurrently with moderate-dose furosemide, would activate the RAAS more than furosemide alone at the same dose.

Twelve healthy dogs, randomized into groups of six, received furosemide (2mg/kg PO q12h) for 10 days (Group 1) or furosemide (2mg/kg PO q12h) and pimobendan (0.6mg/kg PO q12h) for 10 days (Group 2). Heart rate, body weight, blood pressure, and urine aldosterone:creatinine (UA:C, a measure of circulatory RAAS activation) were measured on days -2,-1,1,5 and 10. Serum chemistry and CBC were measured on days -2,5 and 10.

Although there was a statistically significant rise in UA:C in Group 2 over the study period ($p = 0.0015$), the increase differed significantly only on day 1 ($p = 0.044$). 'High-dose' pimobendan, therefore, neither suppresses nor potentiates, to a clinically-significant degree, furosemide-induced RAAS in healthy dogs.

## ABSTRACT C-21

**DURATION OF BETA-BLOCKADE ASSOCIATED WITH REPEATED ONCE-DAILY ADMINISTRATION OF ATENOLOL IN HEALTHY DOGS.** MI Waterman, JA Abbott, AC Lantis, JR Wilcke. Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

Atenolol is a β-adrenergic receptor antagonist used in the management of cardiovascular disease. In dogs, dose intervals of 12 and 24 hours are used but the duration of β-blockade associated with oral administration of atenolol is unknown.

To test the hypothesis that repeated, once-daily oral administration of atenolol attenuates the heart rate response to isoproterenol for 24 hours, we performed a double-blind, randomized, placebo-controlled crossover experiment.

Twenty healthy dogs were randomly assigned to receive either placebo (P) and then atenolol (A) [1 mg/kg PO QD] or, vice versa. Treatment periods were 5-7 days; time between periods was 7 days. Heart rates (bpm) at rest ($HR_r$) and during constant rate [0.2 μg/kg/min] infusion of isoproterenol ($HR_i$) were electrocardiographically obtained 0, 0.25, 3, 6, 12, 18, and 24 hours after final administration of drug or placebo. Repeated measures ANOVA was used to evaluate the effects of treatment (Tr), time after drug or placebo administration (t), interaction of treatment and time (Tr*t) as well as period and sequence on $HR_r$ and $HR_i$.

Sequence or period effects were not detected. There was a significant effect of Tr ($p < 0.0001$) and Tr*t ($p < 0.0001$) on $HR_i$. Atenolol significantly attenuated $HR_i$ for 24 hours but did so maximally at $t = 3$ (least squares means ± SE, A: 146±5, P: 208±5); the effect at $t = 24$ was small (A: 193±5, P: 206±5). Atenolol had a small but significant effect ($p < 0.0001$) on $HR_r$. These data may be relevant to the use of oral atenolol in canine cardiovascular disease.

## ABSTRACT C-22

**EFFECT OF KETANSERIN, A SEROTONIN 2A-RECEPTOR ANTAGONIST, ON ECHOCARDIOGRAPHIC INDICES OF MITRAL REGURGITATION IN DOGS WITH DEGENERATIVE MITRAL VALVE DISEASE.** DJ Trafny[1], GE Singletary[1], RJ Levy[2], MA Oyama[1]. [1]University of Pennsylvania, Philadelphia, PA, [2]Children's Hospital of Philadelphia, Philadelphia, PA

Canine mitral valve interstitial cells can be activated by serotonin. A role of serotonin in degenerative mitral valve disease (DMVD) is suggested by various transcriptional, molecular, and biochemical studies in dogs. We sought to determine the effects of ketanserin, an oral serotonin 2A-receptor blocker on echocardiographic severity of mitral regurgitation in dogs with DMVD. Twenty-seven dogs with ISACHC class I or II DMVD were enrolled. Eight dogs completed a phase I open-label 6-month tolerability and dose-escalation study, followed by recruitment of dogs into a phase II randomized, placebo-controlled, double-blinded, 12-month trial. The primary endpoint of the randomized trial was mitral regurgitant fraction (RF) as measured by Doppler echocardiography. Secondary endpoints included 2D-echo mitral valve length, thickness, area, and prolapse, left ventricular and atrial diameter, and width of the color flow regurgitant jet vena contracta. Results of phase I indicated that ketanserin up to doses of 1.0 mg/kg BID were tolerated. Nineteen dogs completed Phase II. Over a median study duration of 392 days (range, 357-419 days), RF increased relative to baseline by 50.8% and 13.8% in dogs receiving 1.0mg/kg BID ketanserin and placebo respectively, and this difference was not statistically significant ($P = 0.21$). There were no significant differences in any secondary endpoint between groups. Chronic oral ketanserin administration did not alter or slow echocardiographic severity of mitral regurgitation in dogs with DMVD. Further studies are needed to determine the importance of the serotonin pathway in the pathophysiology of canine DMVD.

## ABSTRACT C-23

**MYOCARDIAL ICAM1 MRNA IS INCREASED IN CANINE DILATED CARDIOMYOPATHY.** S Fonfara[1], MA Oyama[2], P Cripps[1,3], SR Tew[1], PD Clegg[1]. [1]Department of Musculoskeletal Biology and School of Veterinary Sciences, University of Liverpool, Neston, UK., [2]Department of Clinical Studies, University of Pennsylvania, Philadelphia, PA USA., [3]Department of Epidemiology & Population Health, University of Liverpool, Neston, UK.

The role of intramyocardial inflammation in canine dilated cardiomyopathy (DCM) is unknown. Presence of endothelial intercellular cell adhesion molecule-1 (ICAM1) expression suggests myocardial inflammation, pro-inflammatory cytokines, and leukocyte infiltration. Previously, no association between ICAM1 and canine degenerative valvular disease was detected but the potential role of ICAM1 in DCM is unknown.

ICAM1-gene expression in myocardial samples (right and left atrium and ventricle) of eight healthy dogs, four DCM dogs, and five dogs with other cardiac diseases was investigated by quantitative RT-PCR. The association between ICAM1 with myocardial cytokine expression (IL1, IL2, IL4, IL6, IL8, IL10, TNFα, IFNγ) was determined.

There was no difference in ICAM1 mRNA between cardiac regions ($p = 0.08$). However, a significantly higher ICAM1 mRNA expression was present in dogs with DCM in comparison to control dogs and dogs with other cardiac diseases ($p = 0.004$). Dogs with cardiac diseases and with and without congestive heart failure (CHF) showed no significant difference ($p = 0.16$), suggesting an increase of ICAM1 mRNA in DCM but not necessarily other cardiac diseases causing CHF.

A positive correlation of ICAM1 with pro-inflammatory IL1, IL6, TNFα and anti-inflammatory IL10 was present ($p < 0.001$). The cytokines were also elevated in dogs with DCM in comparison to dogs with cardiac diseases other than DCM ($p < 0.01$). These results suggest that activation of the inflammatory system and increased ICAM1 expression might be associated with canine DCM.

## ABSTRACT C-24

**ASSOCIATIONS BETWEEN N-TERMINAL PROCOLLAGEN TYPE III (PIIINP), FIBROSIS AND ECHOCARDIOGRAPHIC INDICES IN DOGS WITH MITRAL VALVE DISEASE.** MJ Hezzell[1], T Falk[2], LH Olsen[2], A Boswood[1], J Elliott[1]. [1]The Royal Veterinary College, London, UK, [2]University of Copenhagen, Denmark.

PIIINP is a serum biomarker of collagen biosynthesis and is described as a marker of myocardial fibrosis in human patients.

We hypothesised that PIIINP concentrations would increase with increasing myocardial fibrosis in dogs with MMVD.

Myocardial tissue samples were collected post mortem from the following sites; upper and lower cranial and caudal papillary muscle, upper, middle and lower left ventricular wall, inter-ventricular septum and the right ventricular wall. Between four and eight tissue sections from each myocardial site were examined by a pathologist masked to the dog's clinical history. Myocardial fibrosis was scored as follows: 0, none; 1, mild interstitial; 2, moderate interstitial; 3, small to moderate areas of confluent fibrosis; 4, large areas of confluent fibrosis. The average fibrosis score for all sites in the heart (up to 72 sections) was designated the global fibrosis score (GFS). The average fibrosis score for all papillary muscle sites (up to 36 sections) was designated the papillary fibrosis score (PFS). Serum PIIINP concentrations (μg/ml) were measured using a validated commercially-available radioimmunoassay. Echocardiographic measurements and serum PIIINP are reported for the last examination prior to death or euthanasia in all cases. Non-normally distributed variables were logarithmically transformed. Correlations were assessed using Pearson's correlation coefficient. Univariate linear regression analyses were used separately to evaluate associations between GFS and PFS, respectively, and the following characteristics: PIIINP, left atrial to aortic root ratio (LA/Ao), left ventricular end diastolic diameter to left ventricular free wall thickness in diastole ratio (LVEDD/LVFWd) and left ventricular end diastolic diameter normalised for body weight (LVEDDN). Variables significant at the 20% level were entered into multivariable linear regression analyses with GFS and PFS as the dependent variable in turn.

Twenty-two dogs with MMVD were studied. In the univariate analysis (dependent variable; GFS), PIIINP ($P = 0.074$) and LVEDDN ($P = 0.180$) were associated with GFS. In the final multivariable model, LVEDDN ($P = 0.044$, $B = 1.264$) and PIIINP ($P = 0.020$, $B = 0.061$) were independently positively associated with GFS ($R^2 = 0.318$). In the univariate analysis (dependent variable; PFS), PIIINP ($P = 0.092$), Log (LA/Ao) ($P = 0.076$), LVEDDN ($P = 0.097$) and LVEDD/LVFWd ratio ($P = 0.175$) were associated with PFS. In the final multivariable model, LVEDDN ($P = 0.019$, $B = 1.628$) and PIIINP ($P = 0.018$, $B = 0.068$) were independently positively associated with PFS ($R^2 = 0.289$).

In conclusion, both LVEDDN and serum PIIINP increase with increasing fibrosis score. The relationship between extracellular matrix turnover, fibrosis and left ventricular remodelling appears to be complex in dogs with MMVD.

## ABSTRACT C-25

**NOT ALL ARTERIES ARE CREATED EQUAL: COMPARISON OF PRESSURES WITHIN THE AORTIC ROOT AND THE DORSAL PEDAL ARTERY IN CATS.** K Parker, A Carr, T Duke-Novakovski. Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK.

Blood pressure measurement is important when assessing hemodynamic stability in cats. Any accessible artery, including the dorsal pedal (DP), has been considered an appropriate artery for direct measurement of systemic blood pressure.

To examine measurement of blood pressure within the dorsal pedal artery compared to within the aortic root.

Five anesthetized cats had a 24 gauge catheter with an external pressure transducer (AD Instruments) placed in DP. A transducer tip catheter (3.5 Fr. Millar) was then placed in the carotid artery and advanced to approximately the sixth intercostal space, the level of the aortic root. These were used for continuous blood pressure monitoring. Data were recorded using Powerlab (Powerlab 26T, Model ML856) LabChart7.2 software (AD Instruments). Topical prilocaine and lidocaine cream was applied prior to surgical cut down to the DP artery to minimize vascular spasm. The DP and aortic root catheters were calibrated using a mercury sphygmomanometer prior to placement. Systolic blood pressure was adjusted using various isoflurane concentrations, colloids and dobutamine to achieve hypo- (<80 mmHg), normo- (81-110 mmHg) and hypertension (>110 mmHg). Bias (DP minus Aortic Root), precision (SD of the differences) and limits of agreement (bias ± 2SD) were calculated.

551 matched pairs of data were compared to the aortic root in hypo-, normo-, and hypertensive states for Systolic, Diastolic and Mean values, DP was inaccurate with a bias of(-22.2) for hypotensive systolic (SD 16.6, Correlation Coefficient (CC) 0.74), (-22.9) hypotensive diastolic (SD 12.7, CC 0.73), (-22.2) hypotensive mean (SD 13.4, CC 0.75), (-30.4) normotensive systolic (SD 17.8, CC 0), (-28.0) normotensive diastolic (SD 12.8, CC .49), (-28.4) normotensive mean (SD 12.8, CC 0.58), (-39.8) hypertensive systolic (SD 14.1, CC 0.78), (-29.13) hypertensive diastolic (SD 16.3, CC 0.66), and (-31.6) hypertensive mean (SD 16.7, CC 0.66).

Using the dorsal pedal artery with a 24 GA catheter and external pressure transducer is not an accurate method to measure systemic arterial blood pressure in cats.

## ABSTRACT C-26

**RETROGRADE CORONARY VENOUS STEM CELL DELIVERY: A PILOT STUDY EVALUATING SAFETY AND FEASIBILITY.** B Pogue, AH Estrada, HW Maisenbacher III, A Shih, TJ Conlon.

In human medicine, cellular transplantation of allogenic adult stem cells into failing heart muscle has been employed via numerous delivery methods with success, causing reported improvements in heart function and survival for patients with ischemic heart disease. The purpose of this study was to develop a method of stem cell delivery for Doberman Pinschers with Dilated Cardiomyopathy (DCM) in order to determine the (acute) safety and feasibility of delivery of a large number of cells to a large portion of the left ventricular myocardium. Retrograde coronary venous delivery of adipose-derived mesenchymal stem cells transduced with tyrosine mutant Adeno-associated virus 2 (tmAAV2) to express stromal derived factor-1 (SDF-1) was performed in fifteen Dobermans with DCM (12 pre-CHF and 3 with stablized CHF).

A balloon wedge pressure catheter was placed within the coronary sinus from the right jugular vein under general anesthesia. With the balloon tip inflated, saline was injected retrograde to coronary venous flow at a pressure of 300mmHg in order to disrupt the vascular endothelium. Cellular implantation (10 million cells within 20mL of saline) was performed in a similar fashion and the balloon remained inflated for 10 minutes to retain cells within the coronary venous system. Anesthesia time ranged from 33 to 95 minutes (median 45 minutes), and actual procedural time ranged from 30 to 82 minutes (median 35 minutes). Fourteen dogs were discharged 24 hours following the procedure without complications. One dog developed worsening ventricular arrhythmias and suffered cardiac arrest. 7/15 dogs did not return for follow-up as scheduled and 4/8 dogs who did return for follow up eventually succumbed to either sudden death or congestive heart failure (1 at 24 hours as mentioned above, 2 at 8 months, and 1 at 11 months). Antibody titers against tm-AAV2 were measured in 3/15 dogs at baseline and at 1 month and found to be negative at both time points. Echocardiograms were performed at baseline and 24 hours (15/15), at 1 month (8/15), 3 months (5/15), 6 months (5/15), and 12 months (1/15) following cellular implantation. Measurements of ejection fraction (EF), fractional shortening (FS), end diastolic and systolic volume indexes (EDVI, ESVI) were compared to baseline. Statistical comparisons were made using a repeated measures ANOVA. Differences between the means were considered significant at $P > 0.05$. At 1 month, EDVI was decreased compared to baseline (105.97 mL/m$^2$ vs 111.59 mL/m$^2$, $P = 0.017$) and FS was improved compared to baseline (18.67% vs 17.14%, $P = 0.05$). At 3 months, ESVI was increased compared to baseline (79.60 mL/m$^2$ vs 73.76 mL/m$^2$, $P = 0.035$). At 6 months, both ESVI and EDVI had increased significantly (131.2 mL/m$^2$ and 90.02 mL/m$^2$, respectively, $P < 0.0001$ for both).

In summary, retrograde venous delivery of tm-AAV2-SDF1 adipose-derived mesenchymal stem cells appears safe but long term functional effects and changes in expected outcomes for affected dogs were not observed in the few Dobermans in which long term follow up was available.

**ABSTRACT C-27**

**THE NATURAL ANTIOXIDANTS POMEGRANATE EXTRACT AND SOY ISOFLAVONES FAVOURABLY MODULATE CANINE ENDOTHELIAL CELL FUNCTION.**

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Cardiovascular disease, preceded by valvular and vascular endothelial dysfunction, is a prominent cause of death in dogs. Endothelial dysfunction is characterized by impairment of crucial cell functions, e.g. accelerated apoptosis and increased proliferation of endothelial cells. This study aimed to identify and characterize vasoprotective agents which may be able to prevent or ameliorate the sequelae of endothelial dysfunction and subsequent cardiovascular disease in dogs.

Due to the lack of canine-specific data from *in vitro* models, we established such a model on the basis of canine macrovascular (aortic) endothelial cells (CnAoECs), and showed it to be well suited to test both vasoprotective and detrimental agents. As test substances, the natural antioxidants Pomegranate Extract, Soy Isoflavones, Taurine and L-Carnitine were studied in the respective model in comparison to Glutathione (GSH), N-Acetylcystein (NAc), free fatty acids and Pioglitazone (PIO), which were previously shown to modulate human endothelial cell apoptosis and proliferation *in vitro*, mimicking the *in vivo* situation in humans. CnAoECs were cultured on fibronectin coated cell culture dishes in CECGM at 37°C/5%$CO_2$, and were used in passages 5-7. After replating, cells were labeled with $^3$H-thymidine, exposed to the above mentioned test or control agents for 48 h, and apoptosis and proliferation were measured according to standardized protocols.

Pomegranate Extract and Soy Isoflavones, even at the concentration of 50 µg/ml, markedly reduced proliferation of CnAoECs by 90% and 25%, respectively. This effect was dose-dependent. Exposure of CnAoECs to 250 µg/ml of these two substances led to an impressive inhibition of the cells' proliferation to 3.75% and 15.5% of control (set to 100%), whereas PIO and GSH reduced proliferation to only 62.25% and 54.75% of control, respectively.

Pomegranate Extract significantly reduced apoptosis at all concentrations tested. Once again this was in a dose dependent fashion, showing the most striking effect at 250 µg/ml with a reduction of 73% compared to control cells, whereas the anti-apoptotic effect of Soy Isoflavones was observed only for the highest concentration tested (250 µg/ml).

In the course of the present study we established a canine endothelial cell *in vitro* model, very well suited to the standardized testing of potential vasoprotective agents. Our data clearly demonstrate that Pomegranate Extract and Soy Isoflavones dramatically reduce proliferation and apoptosis of canine aortic endothelial cells, which is consistent with a vasoprotective activity for these natural antioxidants. A combination of these substances in association with cardio protective agents acting on other aspects of the cardiac disease process, could be an interesting potential dietary strategy to reduce the progression of canine cardiovascular diseases.

**ABSTRACT C-28**

**POMEGRANATE EXTRACT COMBINED WITH OTHER NATURAL ANTIOXIDANTS PROTECTS CANINE ENDOTHELIAL CELLS FROM DEATH INDUCED BY OXIDATIVE STRESS.**

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Oxidative stress induces damage to canine and human cardiovascular cell types, and is implicated in the initiation and progression of cardiac diseases. Alterations in antioxidant reactivity in dogs' blood can be related to the severity of cardiac insufficiency. Decreased cardiac efficiency contributes to further increased oxygen demand and increased free radical production. The resulting oxidative damage to the endothelium also reduces nitric oxide availability, limiting the ability of the vascular wall to relax and placing further strain on the failing heart. Several natural antioxidant substances have been shown to be beneficial in humans. However their benefit in canine cardiovascular cells has never been addressed.

In order to evaluate this point, we initially developed a canine aortic endothelial cell (CnAoEC)-based model of oxidative stress-induced cytotoxicity. We first validated the use of the MTT assay to assess cell viability after a cytotoxic challenge. We then established that a 24 hour oxidative challenge with 2mM $H_2O_2$ resulted in a reproducible and consistent decrease of cell viability.

In the second part of the study, we used this model to assess the ability of 4 natural substances (Pomegranate Extract, Soy Isoflavones, Taurine and L-Carnitine), alone and in combination, to protect the CnAoECs from oxidative stress. They were also assessed, at 1, 50 and 250µg/ml, for their antioxidant properties using two common acellular *in vitro* free radical scavenging assays.

In both the free radical scavenging assays, Soy Isoflavones and, in particular, Pomegranate Extract, alone or in combination with the other substances, proved to be potent antioxidants. Indeed, 50 µg/ml of Pomegranate Extract was sufficient to quench over 97% of the radicals. Soy Isoflavones quenched 83% and 92% of the radicals at 50 and 250µg/ml respectively. Taurine and L-Carnitine provided only low quenching. However they did not interfere with the action of the Pomegranate Extract and Soy Isoflavones in the mix.

In the CnAoEC assay, Pomegranate Extract showed a strong protective effect and increased the CnAoEC viability by 35% and 82% at 50 and 250µg/ml respectively. In this assay the other substances were not efficient at protecting the cells from such a potent oxidative challenge on their own. However they did not interfere with the beneficial effects of the Pomegranate Extract and may even have had a positive impact on the cells when used in combination (cell viability increased by 41% with the mix at 50µg/ml, which contains only 12.5µg/ml of Pomegranate Extract).

This study has shown that potent natural antioxidant substances such as Pomegranate Extract are able to protect CnAoECs from significant oxidative challenge, representing a potentially beneficial dietary strategy to maintain endothelial cell health during initiation and progression of canine cardiovascular diseases.

**ABSTRACT C-29**

**CHANGES OF SERUM CARDIAC TROPONIN I CONCENTRATION IN 13 DOGS WITH INTRACRANIAL DISORDERS.**

J Kim, R Song, D Lee, H Lee, J Park, C Park. College of Veterinary Medicine, Chonbuk National University, Jeonju, Jeonbuk, Korea.

Cardiac troponin I (cTnI) that is found within myofibers and is leaked from damaged myocardial cell is a sensitive and specific biomarker for myocardial injury in humans and animals. The protein structure of cTnI is highly conserved between species. cTnI has greater sensitivity and specificity for myocardial damage than other test including CK-MB, cardiac troponin C and T.

Seizures can result in myocardial damage through elevated catecholamines causing increased heart rate, blood pressure, contractility, and myocardial oxygen demand.

Serum cTnI concentration was measured by electrochemiluminescence immunoassay (ECLIA) in 13 dogs with seizure caused by intracranial lesions including granulomatous meningoencephalitis (GME), necrotizing meningoencephalitis (NME), glioma, idiopathic epilepsy, arachnoid cysts and head trauma. The serum cTnI concentration was significantly increased in 6 of 13 dogs (reference range 0 to 0.026).

Measuring cTnI concentrations in dogs with seizure caused by intracranial disorders may help to identify myocardial damage and possibly lead to earlier therapeutic interventions.

**ABSTRACT C-30**

**C-REACTIVE PROTEIN IS ELEVATED IN DOGS IN SEVERE HEART FAILURE.** A Domanjko Petrič, T Lukman, B Verk, A Nemec Svete. Clinic for Surgery and Small Animal Medicine, University of Ljubljana, Slovenia.

C-reactive protein (CRP) is an acute phase protein and an early marker of inflammation. Recent studies in human medicine provide evidence that inflammation plays a role in the pathogenesis of cardiovascular disease. To our knowledge, studies that investigate CRP concentration in heart failure in dogs have not been reported. The authors aimed to investigate whether CRP is elevated in patients with various cardiac diseases and heart failure. In addition we were interested whether there is an association between ISACHC class and CRP concentration.

Thirty nine client-owned cardiac patients (31 males and 8 females; 0.8 - 16 years old) and 12 healthy controls (2 males and 10 females; 1 - 10 years old) were included in the study. The dogs were judged to be healthy based upon normal history, clinical examination, and results of hematological and biochemical analysis. Various congenital and acquired cardiovascular diseases were confirmed on the basis of history, clinical examination, thoracic radiographs, electrocardiogram and echocardiography and classified into International Small Animal Cardiac Health Council classes (ISACHC). Patients with other diseases were excluded from the study. Serum CRP concentration was determined with LifeAssays® Canine CRP Test Kit, which is a two-site heterogeneous immunoassay. Independent T-test was used to compare serum CRP levels between controls and individual class of heart disease. One-Way ANOVA was used to test for statistically significant differences in CRP concentration between ISACHC groups. Pearson's correlation coefficient test was used to correlate ISACHC class with CRP concentration, white blood cell count (WBC) and neutrophil cell count (NEUTH). A value of $P < 0.05$ was considered statistically significant. Results of statistical analysis demonstrated significantly higher CRP concentration in ISACHC III group (range: < 10 - 62 mg/L) versus controls (range: < 10 – 13 mg/L), while no significant differences were found between controls and ISACHC I and II. Serum CRP was significantly higher in ISACHC II (range: < 10 -37 mg/L) and III versus ISACHC I (< 10 mg/L) and significantly higher in ISACHC III comparing to ISACHC II. Significant positive correlations were found between ISACHC class and CRP ($r = 0.330$; $P = 0.040$), WBC ($r = 0.459$; $P = 0.000$) and NEUTH ($r = 0.464$; $P = 0.000$), as well as between CRP and WBC ($r = 0.690$; $P = 0.000$) and between CRP and NEUTH ($r = 0.634$; $P = 0.000$).

In conclusion, CRP concentration is elevated in heart failure patients and increases with severity of heart failure. The results of this present study warrant further studies on CRP as a biomarker of effectiveness of cardiac therapy.

**ABSTRACT C-31**

**ASSESSMENT OF HYPERTROPHIC CARDIOMYOPATHY IN SMALL BREED DOGS AFFECTED WITH HYPERADRENOCORTICISM USING 2D SPECKLE-TRACKING ECHOCARDIOGRAPHY.** HY Chen, HP Huang. Institute of Veterinary Clinical Science, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan.

The aim of the study was to assess left ventricular systolic and diastolic function using 2D speckle-tracking echocardiography (2D-STE) in small breed dogs affected with hypertrophic cardiomyopathy (HCM) in pituitary- and adrenal-dependent hyperadrenocorticism (PDH and ADH, respectively) and age-matched control dogs.

Diagnosis of HCM was based upon findings of conventional echocardiography. Diagnosis of hyperadrenocorticism (HAC) was based on affirmative results from adrenocorticotropic hormone stimulation tests and ultrasonographic adrenal morphology. In 2D-STE, peak systolic and diastolic radial/longitudinal tissue velocity, circumferential/longitudinal strain rate and peak systolic circumferential/longitudinal strain of 6 segments, and septal and lateral corners of mitral annulus were assessed from right parasternal short-axis/left apical views.

In conventional echocardiography, 7 dogs with PDH (prevalence: 7/13, 54%), 8 dogs with ADH (8/12, 67%) and 11 non-HAC dogs (11/53, 21%) were diagnosed as HCM ($P = 0.002$). In these dogs, either the systolic (fractional shortening, ejection fraction) or diastolic parameters (E/A ratio, isovolumetric relaxation time) was not different among dogs with PDH, ADH and non-HAC dogs. In 2D-STE analysis, 23 of 98 (23%) 2D-STE variables were significantly different among 3 groups. The dogs with ADH exhibited significantly decreased longitudinal peak systolic and early diastolic strain rates ($P = 0.046$ and $P = 0.002$, respectively), and longitudinal peak systolic strain ($P = 0.038$) compared to PDH and non-HAC dogs.

HCM was prevalent in HAC comparing to age-matched non-HAC small breed dogs. In dogs with HCM, 2D-STE revealed significant decreases in both diastolic and systolic functions those were not detected using conventional echocardiography in ADH comparing to PDH-affecting and non-HAC dogs.

**ABSTRACT C-32**

**ECHOCARDIOGRAPHIC EVALUATION OF LEFT ATRIAL SIZE IN HEALTH COCKER SPANIEL DOGS AND WITH DILATED CARDIOMIOPATHY.** GT Goldfeder, PH Itikawa, GG Pereira, JR Castro, VM Oliveira. MHMA Larsson School of Veterinary Medicine and Animal Sciences, University of Sao Paulo, SP, Brazil.

Left atrial (LA) size is considered an important prognostic factor in many cardiomyopathies. In humans, it is known that LA remodeling can occur in different planes, according to the cause. The aim of this study was to evaluate left atrial size, by measuring the apical-basilar (ABd), medio-lateral (MLd) and craniocaudal (CCd) axis.

Eleven healthy adult Cocker Spaniel dogs (control) and nine adult Cocker Spaniel dogs with dilated cardiomiopathy (DC) were included. Control animals were screened by physical examination, thoracic radiography, electrocardiography, systolic blood pressure measurement, echocardiography and laboratory tests (CBC, biochemical blood urea and creatinine). ABd and MLd measurements were done from apical 4 chamber view (Ap4cv) of the heart, in end-systole. ABd was obtained by drawing a line from the mitral apposition point of mitral leaflets to middle point of LA roof. MLd measurement was performed drawing a perpendicular line to the ABd. CCd measurements were done anterior-posterior diameter from the paraesternal long-axis view. Data were tested for normality and expressed as mean and standard deviation. Comparisons among the methods were performed by One-way Analysis of Variance.

LA measurements in ABd were 2.63±0.21 cm to control and 4.34±0.83 cm to DC; in MLd were 2.31±0.18 cm to control and 3.57±0.65 cm to DC; in CCd were 2.44±0.21cm to control and 3.59±0.42cm to DC. LA diameters were significantly different among methods. Posterior similar studies involving dogs with different heart disease are recommended.

**ABSTRACT C-33**

**TIME-DOMAIN HEART RATE VARIABILITY IN HEALTHY DOGS.** E Zacché, FN Gava, EMG Ortiz, FA Rosa, R Camacho, FM Marinho, R Navarrete, AA Camacho. São Paulo State University – UNESP, Jaboticabal, São Paulo, Brazil.

Heart rate variability (HRV) is one of the most valuable non-invasive marker of autonomic nervous system and is a prominent method to assess its modulation of cardiovascular system. It is well known that, besides different diseases, several physiologic conditions can affect HVF in human beings but it still being studied in canine patients. Thus, the aim of the present work was to evaluate the influence of different characteristics in HRV in healthy dogs.

Twenty client-owned healthy dogs of different breeds were enrolled in the study. Heart rate variability, determined from 24-hour Holter recordings in home environment, was assessed by SDNN, SDANN, SDNNindex, rMSSD and pNN50. Dogs were grouped on the basis of different criteria such as gender (male, $n = 8$ and female, $n = 12$), age ($\leq 3$ years-old, $n = 7$ and $> 3$ years-old, $n = 13$), cranial conformation (brachycephalic, $n = 5$ and

non-brachycephalic, n = 15) and body weight ($\leq$ 15 Kg, n = 11 and > 15 Kg, n = 9). Data was submitted to a normality test and then analysis between groups was performed by using unpaired Student's t-test.

There was no significant difference (p > 0,05) between groups when SDNN, SDANN, SDNNIDX and rMSSD were evaluated on the basis of any of the tested criteria. The general mean (± standard deviation) of such parameters were 328 ± 85 ms, 164 ± 35 ms, 277± 85 ms and 130 ± 44 ms, respectively. However, pNN50 was significantly higher (p = 0,02) in patients with body weight $\leq$ 15 Kg (71,93 ± 8,22 %) comparing with patients with body weight 15 Kg (62,66 ± 9,13 %).

Indexes based on the comparison between adjacent cycles (rMSSD and pNN50) reflect, predominantly, vagal tonus. Thus, presented data strongly suggest that parasympathetic nervous system of healthy dogs is not influenced by age, gender or cranial conformation but it is body weight dependent. For this reason, the establishment of normal range values should be based especially on that last criteria.

**ABSTRACT C-34**
**HEART RATE VARIABILITY IN BOXER DOGS WITH ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY.** PPC Chamas, VMC Oliveira, FL Yamaki, MHMA Larsson. School of Veterinary Medicine and Animal Science – University of São Paulo, SP, Brazil.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a familial disease that affects mainly Boxer dogs, characterized by frequent ventricular arrhythmias that lead to sudden death. In human patients, studies suggest important role of sympathetic overstimulation in the genesis of these ventricular arrhythmias through observation of diminished heart rate variability (HRV) indices.

Seventy-three Boxer dogs were screened and classified, accordingly to results of ambulatory electrocardiography and echocardiography, into four groups: CB - control Boxers (n = 28, less than 50 VPC/24 hours), SB – suspected Boxers (n = 8, 50 to 300 VPC/24 hours), ARVCB – affected Boxers without systolic dysfunction (n = 19, more than 300 VPC/24 hours) and SDB – affected Boxers with systolic dysfunction (n = 18). Five time domain HRV indices were analyzed: SDNN, SDANN, SDNNi, rMSSD and pNN50. Statistical analyses comprised ANOVA test, Pearson correlation and Cox proportional hazards regression.

Compared to CB group, there was no decrease in HRV indices on SB or ARVCB groups, and no correlation was found between HRV indices and number or degree of ventricular arrhythmias, suggesting that ventricular arrhythmias in Boxer ARVC do not seem to be caused by increased activity of sympathetic nervous system, as observed in human patients. Otherwise, SDB group had impairment of these indices, justified by sympathetic stimulation that occurs in congestive heart failure (CHF) associated with systolic dysfunction. No relation was found between HRV indices and cardiac related death (sudden death or death due to CHF) of dogs with ARVC, demonstrating that such indices are poor prognostic indicators for this disease.

**ABSTRACT C-35**
**RADIOGRAPHIC VERTEBRAL HEART SIZE AND LEFT ATRIAL BISECTING LINE: INTEROBSERVER VARIABILITY AND COMPARISON TO ECHOCARDIOGRAPHIC LEFT ATRIAL SIZE IN DOGS WITH DEGENERATIVE MITRAL VALVE DISEASE.** J Hernandez-Lopez, MC Machen, MA Oyama. University of Pennsylvania, Philadelphia, PA

Radiographic assessment of left heart enlargement is an important diagnostic component of canine degenerative mitral valve disease (DMVD). Previously, investigators have proposed the radiographic left atrial bisecting line $^{LABL}$ as a more specific measure of left atrial enlargement than vertebral heart size (VHS).

Radiographs from 23 dogs diagnosed with DMVD from 2009-2011 were examined by three investigators and VHS and LABL (defined as the line from the 90 degree intersection of the short and long axis VHS measurements to the dorsal border of the left atrium) were measured in vertebral bodies. Interobserver variability (coefficient of variation, CV) and correlation to echocardiographic LA:Ao were calculated.

CV of right and left lateral LABL (R-LABL, 15.3%±13.3%; L-LABL, 25.1%±23.1%) were significantly (P < 0.0001) greater than CV of VHS (R-VHS, 6.1%±4.4%; L-VHS, 4.2%±4.3%). Linear regression revealed moderate correlation between R-VHS ($r^2 = 0.406$; P = 0.006) or L-VHS ($r^2 = 0.551$; P = 0.004) and LA:Ao and weak or non-significant correlation between R-LABL ($r^2 = 0.265$, P = 0.034) or L-LABL ($r^2 = 0.208$; P = 0.12) and LA:Ao. Median R-or-L-LABL was not significantly (P > 0.05) greater in dogs with CHF (n = 12) vs. without CHF (n = 11). Difficulty of the LABL measurement was graded as easy, moderate, or hard in 54%, 28%, and 18% of measurements, respectively.

Assessment of left atrial size using LABL is challenging due to high interobserver variability and weak correlation with echocardiographic LA:Ao, which is likely due to difficulty in ascertaining the dorsal radiographic border of the left atrium in dogs with more advanced disease. Future studies evaluating modifications of the LABL technique should be performed.

**ABSTRACT C-36**
**LEFT ATRIAL FUNCTION ANALYSIS BY LEFT ATRIAL TRACKING METHOD IN CANINE CHRONIC MITRAL VALVULAR HEART DISEASE.** K Nakamura, T Osuga, M Yamasaki, H Ohta, M Takiguchi. Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.

Left atrial tracking method is a novel echocardiographic modality for evaluation of left atrial function. The purpose of this study was to assess the value of this method for the evaluation of canine chronic mitral valvular heart disease.

Firstly, the repeatability of variables of left atrial tracking method was investigated with 6 normal beagles. The within-day and between-day coefficients of variation of %LASpass (conduit function index), %LASact (booster pump function index), %LAStotal (reservoir function index) were all clinically acceptable (< 20%).

Secondly, 36 clinical dogs with chronic mitral valvular heart disease (15 dogs in ACVIM stage B1, 9 dogs in stage B2, 6 dogs in stage C, 6 dogs in stage D) were enrolled. Receiver operating characteristic curve analysis revealed that LA/Ao, E, %LASact were highly useful (area under the curve > 0.9) and E/A, E/E', E'/A', %LAStotal were moderately useful (area under the curve > 0.7) to distinguish asymptomatic dogs (stage B1 and B2) from symptomatic dogs (stage C and D). On the other hand, only % LASact was highly useful (area under the curve > 0.9) and E, E/A, %LASpass, %LAStotal were moderately useful (area under the curve > 0.7) to distinguish mild symptomatic dogs (stage C) from severe symptomatic dogs (stage D). LA/Ao had no value to distinguish mild from severe symptomatic dogs.

In conclusion, these results indicate that left atrial tracking method has adequately high repeatability for routine clinical usage in dogs. Moreover, this method is more valuable than conventional echocardiographic method in assessing the severity of canine chronic mitral valvular heart disease.

**ABSTRACT C-37**
**EARLY DETECTION OF LEFT VENTRICULAR DYSFUNCTION IN DOGS WITH DOXORUBICIN-INDUCED CARDIOMYOPATHY BY 2D SPECKLE TRACKING ECHOCARDIOGRAPHY.** K Sugimoto, H Takano, H Sunahara, T Aoki, G Sugahara, Y Une, Y Fujii. Azabu University, School of Veterinary Medicine, Kanagawa, Japan.

Doxorubicin (DOX) is one of the most frequently used drugs for chemotherapy in dogs, and well known to have cardiac toxicity. Two-Dimensional Speckle Tracking Echocardiography (STE) is a relatively novel method to assess regional myocardial function and has been reported to be useful to detect early myocardial dysfunction in doxorubicin-induced cardiomyopathy of human and rats. The purpose of the study is to evaluate the usefulness of STE to detect early left ventricular (LV) myocardial dysfunction due to DOX in dogs by comparing the changes of STE parameters with histopathological findings of corresponding regions in LV.

DOX (30 mg/m$^2$, 3 weeks apart, 4 times, total 120 mg/m$^2$) and saline were administered to five (DOX group) and four clinically healthy beagles (placebo group), respectively. Conventional echocardiographic and STE parameters were measured and compared. All dogs were euthanized and the histopathologic score corresponding to echocardiographic regions in LV was measured.

Radial strain at the apical level and radial strain rate in early diastole at chordae tendineae level decreased significantly in the DOX group without significant changes in conventional parameters. Three of 4 dogs in the DOX group appeared to have decreased STE parameters corresponding to severity of histopathological lesions. Histopathological lesions among segments were almost homogeneous in LV, but, changes of segmental STE parameters did not always agree with the histological score. Because of complex arrays of myocardial fiber in some LV regions, STE parameters may not accurately detect localized myocardial lesions in some areas.

**ABSTRACT C-38**

**DOXORUBICIN-INDUCED DILATED CARDIOMYOPATHY IN A RABBIT MODEL: AN UPDATE.** FN Gava, E Zacché, EMG Ortiz, T Champion, MB Bandarra, JC Barbosa, RO Vasconcelos AA Camacho. São Paulo State University – UNESP, Jaboticabal, São Paulo, Brazil.

Dilated cardiomyopathy (DCM) is characterized by chamber dilation, important systolic dysfunction and poor prognosis. Thus, studies with experimental models of DCM are always important. The purpose of this study was to investigate the onset of systolic dysfunction using echocardiography in rabbits receiving different doses of doxorubicin (DOX) and to characterize by histological analysis and scanning electron microscopy from three different areas of myocardium.

Thirty male New Zealand White rabbits were randomized in three experimental groups with 10 rabbits each, named: G1: control group, receiving NaCl 0.9 %, G2: receiving DOX 1 mg/kg twice a week and G3: receiving DOX 2 mg/kg once a week, for 6 weeks. Echocardiographic evaluations were performed in awaken animals, before DOX administration (T0) and every fifteen days (T15, T30, T45 and T60). The statistical analysis was performed by analysis of variance followed by Tukey´s test.

Vacuolisation, intracytoplasmic granulation, necrosis and extensive interstitial fibrosis characterized DCM through histology and electron microscopy in left ventricle (LV), interventricular septum (IVS) and right ventricle (RV). The results showed a high mortality rate (70%) for G3 and only 30% for G2. There were significant reductions ($p < 0.05$) in fractional shortening for G2 and G3 at T45 (G1 = 38.57 ± 2.22%, G2 = 20.71 ± 4.02%, G3 = 22, 66 ± 3.78%) and T60 (G1 = 37.35 ± 4.83%, G2 = 22.66 ± 3.50%, G3 = 22.33 ± 4.04%). This was also detected for the ejection fraction at T45 (G1 = 73.28 ± 3.03%, G2 = 45.78 ± 7.44%, G3 = 49.33 ± 6.80%) and T60 (G1 = 71.00 ± 5.83%, G2 = 49.50 ± 6.25%, G3 = 48.33 ± 6.65%).

Echocardiograph was important to indicate the start of systolic dysfunction whereas the histology and electron microscopy featured the mainly lesions of doxorrubicin-induced DCM. The administration of 1 mg/kg twice a week for only 6 weeks is sufficient to induce DCM in rabbits.

**ABSTRACT C-39**

**ASSESSMENT OF SERUM ADIPONECTIN AND ITS CORRELATION WITH OBESITY AND MITRAL VALVE ENDOCARDIOSIS IN DOGS.** MHMA Larsson[1], FLAM achado[1], DS Schwartz[1], G Goldfeder[1], MM Jerico[2]. [1]School of Veterinary Medicine and Animal Science – University of São Paulo, São Paulo, SP, Brazil, [2]School of Veterinary Medicine - University Anhembi Morumbi, São Paulo, SP, Brazil.

Adipose tissue is known as a site for energy storage and synthesis of hormones. Among them, adiponectin, a protein expressed exclusively in adipocytes, is responsible for the correlation between obesity and the atherosclerosis associated with insulin resistance. Its circulating levels are inversely proportional to body fat increase. Studies have suggested that adiponectin has its effects as an anti-atherogenic and anti-inflammatory molecule. However, human studies have shown that circulating adiponectin values should be interpreted with caution, because high levels of adiponectin have been associated with increase mortality rate in patients with coronary artery disease and heart failure. This study evaluated the serum concentration of adiponectin in dogs, divided into three groups: control (n = 8), with chronic mitral valve disease-dvcm (n = 11) and obese patients with chronic mitral valve insufficiency concomitantly (n = 9). Blood samples were processed by means of laboratory techniques of radioimmunoassay (RIA) and ELISA, verifying the correlation between serum adiponectin levels among the groups. The group of dogs with chronic mitral valve disease was classified according to the ACVIM Consensus Statements of 2009. The results showed no statistical significance (Mann-Whitney test) among the groups, probably due to the small sample (RIE: control/dvcm ($p = 0.264$), control/dvcm and obese ($p = 0.112$) and (ELISA: control/dvcm ($p = 0.283$); control/dvcm and obese ($p = 0.147$)), however, we observed a tendency for decrease in mean and median adiponectin values in dogs with dvcm and obese group compared to the control group, confirming the paradox between adiponectin circulating levels in obesity and heart failure.

**ABSTRACT C-40**

**ARTERIAL BLOOD PRESSURE ASSESSMENT IN NORMAL AND OVERWEIGHT DOGS BY THREE DIFFERENT METHODS.** BC Rodrigues, FN Gava, E Zacché, EMG Ortiz, RDM Junior, T Champion, AA Camacho. São Paulo State University – UNESP, Jaboticabal, São Paulo, Brazil.

The measurement of arterial blood pressure (ABP) is an important clinical parameter that should be performed with precision mainly on the investigation of arterial hypertension. The purpose of this study was to compare ABP values obtained indirectly by Doppler and two kinds of oscilometric methods in normal and overweight dogs.

Thirty health adult dogs were selected after physical examination, echocardiograph and laboratory tests. The study groups comprised fourteen overweight dogs with mean body score index (BSI) of 6.9 and sixteen dogs with ideal BSI (4.8). The systolic blood pressure (SBP) was measured in all animals first by Doppler method (A), followed by oscilometric Dixtal® (B) and after by Pet-MAP® (C). The mean and diastolic blood pressure were measured by B and C. The statistical analysis was performed by analysis of variance followed by Tukey´s test.

There was no significant difference between normal and overweight dogs for SBP, but the values were higher ($p < 0.05$) for Pet-MAP® (Overweight dogs: A = 136.21±5,94 mmHg, B = 141.57±2.80 mmHg, C = 160.92±6.34 mmHg; Ideal BSI dogs: A = 136.00±5,04 mmHg, B = 136.56±3.96 mmHg, C = 163.68±6.31 mmHg). For mean blood pressure, there was no difference between normal and overweight dogs, but significant difference between the methods (Overweight dogs: B = 103.28 ±4.71 mmHg, C = 114.00±4.97 mmHg; Ideal BSI dogs: B = 100.81±3.46 mmHg, C = 112.37±3.37 mmHg). For diastolic blood pressure there were no difference between normal and overweight dogs and no difference between the methods (Overweight dogs: B = 82.35 = ±5.00 mmHg, C = 87.00 ±3.63 mmHg; Ideal BSI dogs: B = 81.68±3.54 mmHg, C = 86.50±2.80 mmHg).

The results suggest that overweight dogs may have normal arterial blood pressure but the method of measurement must be chosen carefully, once that Pet-MAP® may overestimate the systolic and mean blood pressure in normal and overweight dogs.

**ABSTRACT C-41**

**PLASMA LEVELS OF NT-PRO BNP AND VENTRICULAR DIASTOLIC FUNCTION ASSESSED BY PULSED TISSUE DOPPLER ECHOCARDIOGRAPHY IN OBESE DOGS.** PRR Melo, AM Mazini, VMC de Oliveira, GT Goldfeder, CN Duarte, DS Schwartz. FMVZ-USP (School of Veterinary Medicine and Animal Science, University of São Paulo) São Paulo, SP, Brazil.

Considering the controversial results on Nt-proBNP in obese humans, and the lack of studies in obese dogs, we sought to evaluate the possible role of Nt-proBNP as a preclinical marker of

cardiac disease in obese dogs, and the relationship with diastolic function parameters assessed by pulsed tissue Doppler echocardiography (TDE).

Forty-eight obese dogs (BCS8/9 and BCS9/9), and 49 dogs with ideal body condition score (BCS5/9) were selected, matched for gender, breed/size and age. Plasma concentration of Nt-proBNP and left ventricular (LV) myocardial TDE derived systolic (Sm), early (Em) and late (Am) diastolic velocities (apical four-chamber view at the lateral mitral annular region) were assessed.

Obese dogs had higher Nt-proBNP levels compared to lean dogs ($P = 0.043$). Eighty percent of BCS5 and BCS9 dogs had Nt-proBNP < 900pmol/L and 42% of BCS8 dogs were between 900-1800pmol/L. Based on a ROC curve, obese dogs showed Nt-proBNP > 550pmol/L while the non-obese showed Nt-proBNP < 550pmol/L, with 75% sensitivity and 53% specificity ($P = 0.044$). Em was decreased ($P = 0.006$) in obese and Ef/Em was increased in BCS8 ($P = 0.02$) and BCS9 ($P = 0.009$) compared to lean dogs, suggesting relaxation change and increased ventricular filling pressure. Increased Ef/Em ratio was associated to higher Nt-proBNP levels ($P = 0.032$). The similarity of Nt-proBNP between BCS9 and BCS5 dogs, despite the presence of preclinical LV diastolic changes, strengthen the need for further studies, especially when considering obese dogs with heart disease, not included herein, which may need lower Nt-proBNP cut-off values in order to differentiate the presence of heart failure as a cause of respiratory distress.

## ABSTRACT C-42

**EFFECT OF BODYWEIGHT ON ASSESSMENT OF LEFT VENTRICULAR FUNCTIONS USING TWO-DIMENSIONAL SPECKLE-TRACKING ECHOCARDIOGRAPHY.** HY Chen, HP Huang. Institute of Veterinary Clinical Science, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan.

The aim of the study was to investigate the effect of body weight on assessment of left ventricular (LV) functions using two-dimensional speckle-tracking echocardiography (2 D-STE) in 70 clinically healthy dogs.

These 70 dogs were categorized into small ( < 7 kg, n = 35), medium (7 to 20 kg, n = 23), and large ( > 20kg, n = 12) dogs. A complete physical examination, non-invasive blood pressure measurements, electrocardiography, thoracic radiography, conventional echocardiography were performed to exclude possible pre-existing cardiopulmonary disease. All dogs underwent 2D-STE: longitudinal peak systolic and diastolic tissue velocity (LTV), strain rate (LSR) and peak systolic strain (LS) of 6 segments (basal, middle, and apical segments of interventricular septum and left ventricular free wall), and also septal and lateral corners of mitral annulus were measured from left apical long-axis views. Peak systolic and diastolic radial tissue velocity (RTV), circumferential strain rate (CSR) and peak systolic circumferential strain (CS) of 6 segments (cranioseptal, cranial, lateral, caudal, ventral, and septal) were measured from right parasternal short-axis views.

Variables derived from strain rate and strain, peak systolic and diastolic LSR/CSR and peak systolic LS/CS, were not affected by bodyweight among these 3 groups. Variables derived from LV velocity, peak systolic LTV/RTV, peak early diastolic LTV/RTV and peak late diastolic LTV/RTV, were significantly affected by bodyweight ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.004$ and $P = 0.001$, respectively) and positively correlated with bodyweight ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.039$ and $P = 0.001$, respectively).

Variables derived from strain rate and strain were not affected by bodyweight and could amend conventional echocardiographic assessment on LV functions in dogs.

## ABSTRACT C-43

**ECHOCARDIOGRAPHIC FINDINGS IN OBESE DOGS.** DS Schwartz, PRR Melo, AM Mazini, VMC de Oliveira, GT Goldfeder, CN Duarte. FMVZ-USP (School of Veterinary Medicine and Animal Science, University of São Paulo) São Paulo, SP, Brazil.

Obesity has an effect on cardiac dimensions and function in humans, and some changes are difficult to be detected on a routine echocardiographic exam. We sought to evaluate several echocardiographic ratios in order to assess cardiac changes in obese dogs compared to non-obese.

Sixty dogs with ideal body condition score (BCS5/9; control) and 60 dogs with BCS8/9 and BCS9/9 (obese) were included, matched for gender, breed/size and age. All dogs were healthy based on clinical-laboratorial evaluation. Echocardiographic variables [left ventricular wall thickness in diastole (LVWd), interventricular septum thickness in diastole (IVSd), left ventricular diastolic diameter (LVDd), and left atria dimension (LA)] were assessed and indexed to aorta (Ao) diameter and/or LVDd. Diastolic function was assessed by early to late mitral flow velocity (E/A). Left ventricular mass (LVM) indexed to Ao was estimated as LVM (Troy) = 1.05([LVDd + LVWd + IVSd]3-[LVDd]3)g. Systolic arterial pressure (SAP) was assessed by Doppler.

Diastolic function was decreased in obese relative to controls ($P = 0.041$). The ratios LVWd/LVDd ($P = 0.002$), IVSd/LVDd ($P < 0.001$) and (LVWd + IVSd)/LVDd ($P < 0.001$) were increased in obese compared to controls. BCS8 dogs had these variables increased in comparison to BCS5 and BCS9. Ratios IVSd/Ao ($P = 0.014$) and (LVWd + IVSd)/Ao ($P = 0.016$) were greater for obese compared to control dogs. Obese dogs had a trend ($0.05 < P < 0.10$) to increased LVWd/Ao ($P = 0.052$) and to indexed LVM ($P = 0.083$). Systolic arterial pressure, LA/Ao and LVWd/IVSd were similar among groups.

Although the measurements may be within normal ranges, these results show concentric increased LVW thickness as an adaptation to overload caused by obesity in dogs, already contributing to diastolic dysfunction.

## ABSTRACT C-44

**CHARACTERIZATION OF GROSS AND HISTOLOGIC MYOCARDIAL LESIONS IN OBESE DOGS.** E Mehlman, J Boon, J Bright, K Jeckel, C Porsche, M Frye. Colorado State University College of Veterinary Medicine, Fort Collins, CO.

Obesity is the most common nutritional disease in dogs, and it is predicted that prevalence will increase as the number of obese humans continues to rise. Human obesity is associated with left ventricular hypertrophy (LVH), and this structural change increases the risk for heart failure. Unpublished data suggest that canine obesity, in the absence of hypertension, is also associated with LVH; the etiology of this structural change is not known. The objectives of this study were to echocardiographically characterize myocardial structure and function in obese normotensive dogs, and examine postmortem tissues from a separate population for evidence of potential contributors to LVH; namely, triglyceride (TG) accretion, collagen accumulation and myocyte hypertrophy.

Obese (body condition score $\geq$ 7/9) and age-matched lean dogs were recruited for echocardiographic examination using electronic notification of students and employees at the Colorado State University Veterinary Medical Center (CSU-VMC). Only normotensive dogs with unremarkable physical examination findings were subsequently included as subjects. The postmortem study was conducted using myocardium from 4 obese and 12 lean dogs with no history of cardiac disease, submitted for necropsy at the CSU-VMC for reasons unrelated to the present study. Samples of left and right ventricles and the interventricular septum were examined for lipid (oil red O staining, TG quantitation), collagen (Masson's trichrome staining, hydroxyproline quantitation) and myocyte cross sectional area (Image J analysis of H&E stained sections).

Echocardiography revealed increased left ventricular free wall thickness in obese dogs during diastole (obese 9.9 ± 0.4 mm, lean 8.7 ± 0.4 mm; $p = 0.03$) and systole (obese 15.2 ± 0.5 mm, lean 12.9 ± 0.5 mm; $p < 0.01$). Septal thickness and left ventricular chamber diameter were similar between groups. Isovolumic relaxation time, a measure of diastolic function, was prolonged in 37% (7/19) of obese dogs. Fractional shortening, an indicator of systolic function, was similar between groups. Tissue studies revealed comparable lipid and collagen content, as well as myocyte area, in lean and obese groups.

The findings of this study support the idea that canine obesity is associated with a focal increase in left ventricular thickness

and possibly diastolic dysfunction, similar to early changes observed in obese humans. Though this study did not reveal a key contributor to gross LVH, trends toward increased TG content and myocyte area observed concomitantly with high variability within groups suggest that larger studies are warranted, controlling for known confounders in humans such as diet, activity and chronicity.

**ABSTRACT C-45**
**TRANSVENOUS PERMANENT PACEMAKER THERAPY IN DOGS: BRAZILIAN EXPERIENCE.** DS Schwartz, DT Fantoni, GT Goldfeder, MHMA Larsson. FMVZ-USP (School of Veterinary Medicine and Animal Science, University of São Paulo) São Paulo, SP, Brazil.

The use of transvenous cardiac pacemaker therapy for bradyarrhythmias is well established in veterinary medicine mainly in the United States and Europe, but it has been introduced into Brazilian veterinary practice only five years ago. During this period, 16 dogs (11 females; 5 males) were treated with transvenous pacemaker. Most dogs (15/16) had syncope as the main complaint, four had pre-syncopal episodes which progressed to syncope and one had seizures episodes and syncope. The most common arrhythmia was complete atrioventricular block (AVB) (62.5%), followed by sick sinus syndrome (31.25%), and one had advanced second degree AVB. The patients included: three Dachshunds, Pitbulls, and Schnauzers; two Labradors; and one Boxer, Bull Terrier, Cocker, English Bulldog and Yorkshire. Age ranged from 2 to 10 years (7.5±2.5 years), with 56.25% over 7 years. Two dogs had the implant as an emergency procedure. Two dogs had decreased ventricular function before the implant. The complications observed (25%) were lead dislodgement (1/16); electrode thrombus formation (1/16); increased impulse threshold after 10 months of implant with decrease in battery lifetime and need for generator replacement (1/16); metastatic implant infection and thrombus (1/16). Eight dogs died in this period (18.14±10.24 months post implant). Causes of death were related to neoplasia (3/8), renal failure (1/8), heart failure secondary to mitral valve disease (1/8), myocardial failure associated with ventricular arrhythmias (1/8), seizures (1/8) and myocardial failure during procedure to change device due to infection (1/8). The type and rate of complications are similar to what is reported in the literature.

**ABSTRACT C-46**
**ECHOCARDIOGRAPHIC EVALUATION OF LEFT ATRIAL SIZE IN MAINE COON CATS, FROM APICAL FOUR CHAMBER VIEW.** PH Itikawa, VM Oliveira, GT Goldfeder, MHMA Larsson. School of Veterinary Medicine and Animal Sciences –University of Sao Paulo, SP, Brazil.

Left atrial (LA) size is considered an important prognostic factor in many cardiomyopathies. In humans, it is known that LA remodeling can occur in different planes, according to the cause. The aim of this study was to evaluate left atrial size, by measuring the apical-basilar (ABd) and medio-lateral (MLd) axis and to compare with standard measurements in M-mode (MM) and in bidimensional LA short axis (2Dsax).

Thirty eight healthy adults Maine Coon cats were included. Animals were screened by physical examination, thoracic radiography, electrocardiography, systolic blood pressure, echocardiography and laboratory tests (CBC, biochemical blood urea, creatinine and total $T_4$). ABd and MLd measurements were done from apical 4 chamber view (Ap4cv) of the heart, in end-systole. ABd was obtained by drawing a line from the mitral apposition point of mitral leaflets to middle point of LA roof. MLd measurement was performed drawing a perpendicular line to the ABd. Data were tested for normality and expressed as mean and standard deviation (± SD). Comparisons among the methods were performed by ANOVA with Repeated Measurements.

LA measurements were: 1.34±0.13 cm in MM; 1.34±0.15 cm in 2Dsax; 1.67±0.18 cm in ABd and 1.49±0.15cm in MLd. LA diameters were significantly different among methods, except between MM and 2Dsax, with higher values obtained from Ap4cv. Posterior similar studies involving cats with different heart disease are recommended.

**ABSTRACT C-47**
**SURVIVAL OF CATS WITH ISOLATED DISCRETE UPPER SEPTAL THICKENING (DUST) VS. CATS WITH AND WITHOUT CARDIOMYOPATHY.** GE Singletary, MA Oyama. University of Pennsylvania, Philadelphia, PA.

Isolated echocardiographic discrete upper septal thickening (DUST) or proximal interventricular septal hypertrophy is occasionally detected in cats but has unknown clinical significance. In humans, isolated DUST does not affect cardiac mortality. We retrospectively evaluated survival in cats with DUST vs. cats with and without cardiomyopathy. We hypothesized that DUST is not associated with increased mortality.

Echocardiograms from cats evaluated during 2009 were segregated into three echo groups, DUST (n = 13), cardiomyopathy (CM, n = 94), or no cardiomyopathy (no CM; n = 44). Median age between groups was significantly different (11.8yrs, 10.4yrs,, 7.6yrs, respectively; $p = 0.004$). Median survival time (MST) between the groups was significantly different (DUST, 513d; CM, 442d; no CM > 800d; $p = 0.046$).

On univariate analysis, age ($p = 0.0001$), CHF ($p < 0.0001$), thromboembolism ($p = 0.025$), and echo group ($p = 0.036$) were significant risk factors for mortality whereas gender was not ($p = 0.06$). Backwards multivariate regression using all variables identified age ($b = 1.09$, CI = 1.04-1.15, $p = 0.0009$), presence of CHF ($b = 5.34$, CI = 3.26-8.72, $p < 0.0001$) and thromboembolism ($b = 2.71$, CI = 1.07-6.86, $p = 0.0361$) as significant risk factors for mortality. Thus, after adjustment for covariates, echo group did not significantly affect mortality.

CM cats were more likely to die of cardiac-related complications vs. cats with DUST or no CM (CM, 35% vs. DUST/no CM, 0%; $p = 0.0006$). Cats with NT-proBNP > 500pmol/L had shorter MST than cats with lower concentrations (> 500pmol/L, n = 9, 314d vs. < 500pmol/L, n = 14, > 786d; $p = 0.009$), however cats with available NT-proBNP results were few. These results indicate that isolated DUST is not a risk factor for mortality and that cats with DUST are unlikely to die from cardiac-related causes.

**ABSTRACT C-48**
**EFFECT OF ATENOLOL THERAPY ON ECHOCARDIOGRAPHIC PARAMETERS IN CATS WITH HYPERTROPHIC OBSTRUCTIVE CARDIOMYOPATHY.** MC Machen[1], MJ Hezzell[2], MA Oyama[1]. [1]University of Pennsylvania, Philadelphia, PA, [2]Royal Veterinary College, London, UK.

Hypertrophic obstructive cardiomyopathy (HOCM) is commonly diagnosed in cats, and consists of left ventricular (LV) concentric hypertrophy and dynamic LV outflow tract obstruction (dLVOTO). Atenolol is commonly prescribed in asymptomatic HOCM, however its effect on LV hypertrophy is largely unknown. We sought to retrospectively evaluate the effects of atenolol on echocardiographic measures of LV hypertrophy and aortic pressure gradient (APG) in asymptomatic cats with HOCM.

A search of medical records from 2005-2011 identified asymptomatic HOCM cats having received atenolol and echocardiographic examinations prior to and after starting atenolol (n = 24). Cats with a diagnosis of non-obstructive hypertrophic cardiomyopathy (HCM) not administered atenolol were identified as controls (n = 12).

Median follow up time was 221d (range, 83-555d). In cats with HOCM, median atenolol dose was 6.25mg/day (range, 6.25mg-18.75mg/day). Repeated measures linear modeling with compound symmetry covariance structure was used to analyze changes in echo variables over time (days). There was a significant difference in the rate of change of interventricular septum in diastole (IVSd) thickness between HOCM + atenolol and HCM cats (P = 0.002), with IVSd significantly decreasing in the HOCM + atenolol group (B = -0.0003, [95% CI, -0.0004 to -0.0001], P < 0.001) but not changing in the HCM group (B = 0.0001[-0.0000 to 0.0004], P = 0.171) (Raw IVSd values: HOCM-pre, median = 0.71cm [IQR, 0.61-0.78cm]; HOCM-post, 0.57cm [0.52-0.66cm] vs. HCM-pre, 0.61cm [0.58-0.63cm]; HCM-post, 0.63cm [0.60-0.70cm]). There was a significant difference in the rate of change of Log[APG] between HOCM + atenolol and HCM cats (P = 0.009), with Log[APG] significantly decreasing in the HOCM + atenolol group (B = -0.0018, [-0.0025 to -0.0012], P < 0.001) but not changing in the HCM group (B = 0.0003[-0.0011 to 0.0017], P = 0.682) (Raw APG values: HOCM-pre, 76.5mmHg [34.4-95.6mmHg]; HOCM-post, 11.2mmHg [4.1-19.9mmHg] vs. HCM-pre, 3.6mmHg [2.6-13.5mmHg]; HCM-post, 4.9mmHg [3.9-10.8mmHg]). There were no significant differences in the rates of change of left ventricular internal diameter in diastole (P = 0.336), left ventricular internal diameter in systole (P = 0.304), left ventricular posterior wall thickness in diastole (P = 0.933), fractional shortening (P = 0.468), or left atrial to aortic root diameter ratio (P = 0.081) between groups.

Atenolol therapy appears to decrease dLVOTO and ventricular septal hypertrophy in cats with HOCM. Limitations of this study include the lack of a true control group and an unknown effect on outcome. Future prospective placebo-controlled randomized studies are needed.

## ABSTRACT C-49
## EFFECT OF A HEART MURMUR ON ADOPTABILITY OF CATS IN AN URBAN NO-KILL RESCUE ORGANIZATION.

RL Cohen, GE Singletary, MC Machen, DJ Trafny, MA Oyama. University of Pennsylvania, Philadelphia, PA.

There are approximately 70 million stray cats in the United States. Many of these cats are rescued and offered for adoption by animal shelter and rehoming organizations, however relatively little is known about the variables that affect the likelihood for successful adoption. Heart disease is an important health issue in adult cats, and we sought to determine the effect of heart murmurs or heart disease on adoptability of cats from a large urban no-kill rescue and rehoming organization.

Adult cats that entered a Philadelphia shelter were enrolled over a 60-day period. Cats < 1 year of age or with serious concurrent medical conditions such as orthopedic injuries or cancer were excluded. A cardiologist and cardiology resident independently ausculted all cats and those found to have murmurs underwent echocardiography. The knowledge of a murmur and subsequent echocardiographic diagnosis were made available to adoption staff and potential adopters. We examined the effect of the presence of a murmur and/or heart disease on adoption outcome (yes vs. no) 90-days after end of study enrollment using logistic regression.

Ninety cats were enrolled, and 10 (11%) were found to have murmurs. Of those, 2 were found to have significant heart disease on echocardiography, while 8 were found to have murmurs of benign origin. At the end of the study, 80 cats had been adopted. On univariate regression, presence of heart murmur (P = 0.18), heart disease (P = 0.49), gender (P = 0.50), and coat color (black vs. other) (P = 0.19) were not associated with adoption outcome. On multivariate regression, no variable was associated with adoption outcome.

Results of this study show that benign heart murmurs are relatively common in shelter cats and the presence of a murmur does not influence adoptability if echocardiographic study indicates normal underlying heart structure. Limitations of the study include relatively low numbers of cats with heart disease. Further studies in larger populations as well as in shelters with different adoption and rehoming policies from those present in this study are warranted.

## ABSTRACT C-50
## DETECTION OF OCCULT FELINE CARDIOMYOPATHY USING A PET-SIDE POINT-OF-CARE NT-PROBNP ELISA ASSAY.

MC Machen[1], SG Gordon[2], JS Buch[3], MJ Beall[3], MA Oyama[1]. [1]University of Pennsylvania, Philadelphia, PA, [2]Texas A&M University, College Station, TX, [3]IDEXX Laboratories Inc., Westbrook, ME

Detection of asymptomatic (occult) feline cardiomyopathy (OCM) is challenging. Previous study indicates that NT-proBNP identifies cats with moderate to severe OCM. We sought to determine the ability of a pet-side point-of-care ELISA that utilizes SNAP® technology and second generation antibodies to detect moderate to severe OCM.

Asymptomatic cats suspected to be "at-risk" for or known to have OCM were prospectively recruited based upon a prior diagnosis, the presence of a heart murmur, gallop, arrhythmia, and/or being a predisposed breed. A group of 16 healthy cats were recruited as controls. All cats underwent physical examination, blood pressure measurement, and echocardiography by an operator blinded to SNAP results. Blood for chemistry profile, T4, and an experimental second generation Cardiopet® proBNP assay was drawn. A SNAP assay was performed at time of echocardiogram and evaluated by both an automated SNAP reader (SNAPshot DX®) and by visual interpretation by a separate reader blinded to the echo results. The SNAP assay result was based on the color of the sample spot compared to the reference spot. SNAP results were called either positive (color of sample spot equal to or darker than the reference spot) or negative (color of sample spot lighter than reference spot).

In addition to the 16 control cats, 48 at-risk cats were examined and classified as normal (n = 14), mild OCM (n = 19), moderate OCM (n = 10), and severe OCM (n = 5). Diagnosis in cats with OCM included HCM (n = 9), HOCM (n = 23), and RCM (n = 2). In the 34 cats with any degree of OCM, SNAPshot DX and visual interpretation of the SNAP had a sensitivity/specificity of 68.8%/91.7% and 68.8%/100%, respectively. In cats with moderate or severe OCM, SNAPshot DX and visual interpretation of the SNAP had a sensitivity and specificity of 100% and 88.2%, and 93.3% and 100%, respectively.

Quantitative ELISA plate assay using second generation antibodies indicated that median plasma NT-proBNP concentration was significantly higher in OCM vs. normal at-risk cats or healthy controls (OCM, 306pmol/L [IQR, 82-690pmol/L]; normal at-risk, 28pmol/L [15-51pmol/L]; control, 31pmol/L [19-58pmol/L]; P < 0.0001). Median NT-proBNP concentration was significantly greater in cats with moderate or severe OCM vs. mild OCM (moderate/severe, 651pmol/L [348-919pmol/L] vs. mild, 115pmol/L [35-286pmol/L]; P = 0.0002). Comparison of results from the SNAPshot DX vs. ELISA plate indicated that a positive SNAP test result was associated with a quantitative NT-proBNP concentration >110-130pmol/L. Results of this study indicate that a point-of-care SNAP assay aligns with ELISA plate assay and detects moderate to severe OCM with high sensitivity and specificity.

## ABSTRACT C-51
## TRICUSPID ANNULAR PLANE SYSTOLIC EXCURSION (TAPSE) MEASUREMENTS IN MAINE COON CATS.

PH Itikawa, GT Goldfeder, VM Oliveira, MHMA Larsson. School of Veterinary Medicine and Animal Sciences –University of Sao Paulo, SP, Brazil.

Assessment of right ventricular (RV) function remains challenging because of complex RV chamber geometry. Recently, in human medicine, many studies have been considered the tricuspid annular plane systolic excursion (TAPSE) a useful tool for RV function evaluation. In that species a good correlation was found

between VD function, obtained by right heart catheterization, and values of TAPSE. RV studies are important for diseases such as pulmonary hypertension and Arrhythmogenic Right Ventricular Cardiomyopathy in cats. The aim of this study was to evaluate TAPSE and to present initial normal reference range for Maine Coon cats.

Twenty eight healthy adults Maine Coon cats were included. Animals were screened by physical examination, thoracic radiography, electrocardiography, systolic blood pressure, echocardiography and laboratory tests (CBC, biochemical blood urea, creatinine and total $T_4$). In face of normal results, TAPSE was obtained by apical four-chamber view and an M-mode cursor was placed through the lateral tricuspid annulus in real time. TAPSE was measured as the total displacement of the tricuspid annulus in (centimeters) from end-diastole to end-systole. Data were tested for normality and expressed as mean, standard deviation (± SD) and 95% confidence interval.

TAPSE measurements obtained were: 0.97 ± 0.25 cm and 0.87 to 1.07 cm, respectively. Posterior similar studies involving more animals, others breeds and cats with different disease are recommended.

**ABSTRACT C-52**

**TRICUSPID ANNULAR PLANE SYSTOLIC EXCURSION (TAPSE) MEASUREMENTS IN DOGS.** GT Goldfeder, PH Itikawa, JR Castro, VM Oliveira, MHMA Larsson. School of Veterinary Medicine and Animal Sciences –University of Sao Paulo, SP, Brazil.

Assessment of right ventricular (RV) function remains challenging because of complex RV chamber geometry. Recently, in human medicine, many studies have been considered the tricuspid annular plane systolic excursion (TAPSE) an useful tool for RV function evaluation. In that species a good correlation was found between VD function, obtained by right heart catheterization, and values of TAPSE. RV studies are important for diseases such as pulmonary hypertension and Arrhythmogenic Right Ventricular Cardiomyopathy. The aim of this study was to evaluate TAPSE and to present initial normal reference range for dogs.

Sixteen healthy adult dogs with 1 to 20 kg (G1) and nineteen dogs with 21 to 40 kg (G2) were included. Animals were screened by physical examination, thoracic radiography, electrocardiography, systolic blood pressure measurement, echocardiography and laboratory tests (CBC, biochemical blood urea and creatinine). In face of normal results, TAPSE was obtained by apical four-chamber view and an M-mode cursor was placed through the lateral tricuspid annulus in real time. TAPSE was measured as the total displacement of the tricuspid annulus in (centimeters) from end-diastole to end-systole. Data were tested for normality and expressed as mean and standard deviation. Comparisons among the groups were performed by unpaired T Test. TAPSE measurements obtained were: 1.22±0.30 cm for G1 and 1.70±0.25 cm for G2. TAPSE measurements were significantly different between groups. Posterior similar studies involving more animals and dogs with different disease are recommended.

**ABSTRACT C-53**

**SURVEY OF SIX POTENTIAL CARDIAC BIOMARKERS IN DOGS WITH HEART DISEASE: CHROMOGRANIN-A, ENDOGLIN, GALECTIN-3, NT-PROBNP, ST2, AND OSTEOPONTIN.** MA Oyama[1], MC Machen[1], DJ Trafny[1], GE Singletary[1], RC Gupta[2], R Sainger[1], HN Sabbah[2]. [1]University of Pennsylvania, Philadelphia, PA., [2]Henry Ford Health Systems, Detroit, MI.

Biomarkers are substances released into the circulation by the injured heart, and have diagnostic and prognostic potential. Other than NT-proBNP, relatively little is known about circulating biomarkers in dogs with spontaneous heart disease. We examined five additional potential biomarkers in dogs with either degenerative mitral valve disease (DMVD) or dilated cardiomyopathy (DCM), and compared results to those from NT-proBNP assay.

EDTA plasma was obtained from 61 healthy dogs, 22 with DCM, and 65 with DMVD (ISACHC classes 1a/b [n = 53], 2 [n = 16], and 3 [n = 18]). Plasma was stored at -80C until batch ELISA analysis. Not all assays were performed in each dog due to the volume of blood required.

Plasma concentrations of chromogranin-A (n = 70), a negative inotrope and marker of sympathetic activation; endoglin (n = 35), a soluble TGF-beta receptor; galectin-3 (n = 50), a marker of fibrosis and remodeling; ST2 (n = 69), a marker of remodeling and cardiac stress; and osteopontin (n = 37), a marker of fibrosis, were not significantly elevated in dogs with heart disease vs. controls (P-values of 0.60, 0.15, 0.21, 0.25, and 0.45, respectively). In contrast, median NT-proBNP concentration was significantly increased in heart disease (2,115pmol/L, n = 87) vs. control (369pmol/L, n = 61; $P < 0.0001$).

Identification of novel biomarkers in dogs is challenging. Limitations of the study include relatively small numbers of dogs with advanced disease and use of ELISA systems that might not be ideally suited for use in canine samples. Future studies might benefit from more efficient high-throughput screening methods to identify candidate markers for further study.

**ABSTRACT C-54**

**BREED-SPECIFIC VERTEBRAL HEART SIZE FOR THE DACHSHUND.** RR Birks, SE Clay, LG Britt, SB Leach, DM Fine. University of Missouri, Columbia, MO.

Heart disease (HD) is common in dachshunds, and is often monitored with radiographs. The radiographic vertebral heart size (VHS) was developed to objectively evaluate the size of the canine cardiac silhouette. A VHS reference range of 9.7±0.5 vertebrae was established using a mixed population of breeds. Subsequent studies have demonstrated that some breeds fall outside this reference range. The aim of this study was to determine a breed-specific VHS for the dachshund and compare results to the established range.

Twenty-four normal control (CON) dachshunds had thoracic radiographs (TXR) and an echocardiogram (Echo) performed. Medical records were reviewed for dachshunds with HD. Twenty-four dogs with a heart murmur, TXR, and an Echo performed on the same day were identified. Five observers of varying levels of clinical training (1 each: senior veterinary student, specialty intern, 3rd year cardiology resident, boarded cardiologist, boarded radiologist) measured VHS for all 48 dachshunds on right and left lateral TXR. Measurements were performed on 2 separate days, at least 1 week apart and were recorded to the nearest 0.25 vertebra. The data was analyzed using Wilcoxon Rank-Sum analysis. The right and left VHS were correlated with Echo 2D long-axis and M-mode left atrial (LA) measurements and M-mode LA to aortic ratio. The Kappa reliability test was used to evaluate inter-observer and intra-observer variability. $P < 0.05$ was considered significant.

The median (range) for the right lateral CON VHS was 9.75 (8.1–11.0). A small but significant decrease in left lateral CON VHS was seen: 9.5 (8.2–10.5). The median right lateral HD VHS measurement was: 11.0 (9–13.5). There was no difference when compared with left lateral HD VHS, 11 (9.2-13.5). The median VHS of HD was significantly greater than that of CON. A high correlation was seen between the VHS and the 2D LA Echo measurements (Right sided VHS, r = 0.824; Left sided VHS, r = 0.817). The Kappa inter-observer and intra-observer variability was 0.69±0.03 and 0.76±0.1 respectively.

Results of this study indicate normal dachshunds have a median VHS within the previously published reference range. A VHS > 11.0 vertebrae may be a reliable indicator for the presence of HD. A significant difference was present between right and left lateral VHS in CON dogs, but not in HD. Therefore, serial VHS measurements should consistently be performed with the dog in the same lateral recumbency to limit variability. Although HD VHS was significantly higher than CON VHS, there was overlap between groups, indicating VHS should be

interpreted in conjunction with clinical and Echo findings. VHS demonstrated a strong correlation with the 2D LA echo measurment. Inter-observer and intra-observer variability showed substantial agreement for measurements of VHS, indicating VHS can be reliably performed by observers with varying degrees of experience.

**ABSTRACT C-55**
**IN-HOME BLOOD PRESSURE MONITORING IN DOGS: A PILOT STUDY.** AP Carr. Western College of Veterinary Medicine, Saskatoon, Canada.

In-home blood pressure (BP) monitoring is commonly used in humans and has been found to be equivalent to 24 hour ambulatory blood pressure (ABP) monitoring. Both ABP and in-home measurements generally eliminate "white-coat" effects as well as detect masked hypertension.

This retrospective study was carried out to see if owners could reliably obtain blood pressure readings in dogs and compare these readings to those obtained in clinic. All in-clinic readings had been obtained in an exam room with the owners present to minimize stress. The readings were obtained using an HDO oscillometric blood pressure device (S + BmedVET, Babenhausen, Germany) while monitoring the oscillometric trace using MDS Analyse software. It was recommended that the owners obtain approximately 5 readings with 3 different BP sessions. All BP traces from in-clinic and in-home sessions were evaluated and readings that showed minimal artifact were used for analysis in this study.

A total of 6 dogs were evaluated that suffered from a variety of clinical problems. Systolic hypertension was present in all 6 dogs in clinic (2 moderate and 4 severe risk of target organ damage or TOD) but only 3 at home (1 mild, 1 moderate and 1 severe risk of TOD). Diastolic hypertension was present in 3 dogs (1 mild and 2 moderate risk of TOD) in clinic and in 1 dog at home (moderate risk of TOD). One dog (CRF, PLN) had pressures obtained twice in clinic and at home with very consistent findings (in clinic: 183/85, HR 221 October 2009, 185/81, HR 207 September 2010; at home: 126/81, HR 109 November 2009, 123/62, HR 108 September 2010). One dog (hyperadrenocorticism) had evidence of masked hypertension (in clinic: 174/95 MAP 123 HR 150; at home: 197/110 MAP 140 HR 80).

In our pilot study all owners were able to obtain blood pressure readings with minimal artifact. Hypertension was diagnosed less frequently at home than in the clinic, though one case of masked hypertension was diagnosed. It appears that in home blood pressure measurement is a viable option for assessing blood pressure in dogs.

**ABSTRACT C-56**
**LONG-TERM SURVIVAL IN DOGS WITH UNTREATED SEVERE SUBAORTIC STENOSIS.** DB Leeder[1], AH Tobias[1], CD Stauthammer[1], SC Hansen[1], TM Hart[2], DM Fine[3]. [1]University of Minnesota, St. Paul, MN, USA, [2]SylvaniaVet, Sylvania, Ohio, USA. [3]University of Missouri, Columbia, MO, USA.

Dogs with untreated severe subaortic stenosis (SAS) are reported to have a poor prognosis. This is largely based on the results of a single retrospective study involving a total of 15 dogs from one institution, which reported median survival of 1.6 years of age. Subsequent studies have evaluated the efficacy of various therapeutic interventions on survival in dogs with SAS; however, none have included an untreated control group. The purpose of this study was to evaluate survival in dogs with untreated severe SAS. The hypothesis was that survival is longer than previously reported. Medical records at the University of Minnesota Veterinary Medical Center and University of Missouri Veterinary Medical Teaching Hospital were reviewed. Dogs with severe SAS confirmed by two-dimensional and Doppler echocardiography ($\Delta P > 80$ mmHg) were identified. Dogs with concurrent hemodynamically-significant cardiac disease and those prescribed ß-blockers or any other cardiac medications at the time of diagnosis were excluded. Between 2002 and 2011, 21 dogs with uncomplicated, untreated severe SAS were identified. Median (range) age and $\Delta P$ at time of diagnosis were 1.4 years (0.2-11.6) and 120 mmHg (81-207), respectively. Kaplan-Meier analysis disclosed that their median survival was 8.8 years of age (95% CI: 4.5-11.8) for an all-cause mortality, with the same median survival for cardiovascular mortality but with a slightly narrower 95% CI (4.9-11.5). Based on these data, we conclude that dogs with untreated severe SAS survive considerably longer than previously reported.

**ABSTRACT C-57**
**COMPARISON OF PIMOBENDAN, BENAZEPRIL, OR METHYLDIGOXIN MONOTHERAPY IN IRISH WOLFHOUNDS WITH OCCULT DCM: A 10-YEAR-STUDY.** AC Vollmar[1], N Mohren[2], C Trötschel[3], PR Fox[4]. [1]Small Animal Veterinary Clinic, Bonn Germany, [2]Boehringer Ingelheim Vetmedica GmbH, Germany, [3]Boehringer Ingelheim Italia S.p. A., Milano, 4. Animal Medical Center, NY, USA.

We prospectively studied cardiac outcome in 66 asymptomatic (ISACHC stage 1B) Irish wolfhound dogs with occult DCM (n = 46) or lone atrial fibrillation (AF) (n = 20). Cases were allocated at random to receive monotherapy with either pimobendan (0.25 mg/kg q12hr, n = 23), benazepril (0.25-0.33 mg/kg/day, n = 22), or methyldigoxin (0.01 mg/kg/day, n = 21) in a single-blinded manner. Concomitant diltiazem (slow release formulation, 1-3 mg/kg q12hr; n = 8) was given for AF HR control. Diagnosis was made by physical examination, echocardiography, chest radiography, and ECG. Dogs were monitored approximately every 6 months. Primary endpoint was CHF or sudden cardiac death (SD). We assessed median time to CHF/SD (Kaplan-Meier analysis) and between-group comparisons (two-sided log rank tests with Bonferroni-Holm multiple comparisons). Significance level was $p < 0.05$.

Median age was 4.0 years, range 1.4-8.0 years (37 male, 27 female. Median weight was 70.0 kg (range 47.6 – 90.0 kg). Median study duration was 52.5 months. Endpoint was reached in 5/23 (21.7%) pimobendan; 11/22 (50%) benazepril; 9/21 (42.9%) methyldigoxin cohorts. Median time to CHF/SD was longer for pimobendan (66.4 months) vs. benazepril (33.2 months) ($p = 0.007$) or methyldigoxin (42.1 months) cohorts ($p = 0.031$).

In this long-term study pimobendan monotherapy improved cardiac outcome for Irish Wolfhounds with occult DCM or lone AF vs either benazepril or methyldigoxin alone.

# EQUINE

**ABSTRACT E-1**
**ECHOCARDIOGRAPHIC ASSESSMENT OF LEFT VENTRICULAR SIZE AND SYSTOLIC FUNCTION IN HORSES USING LINEAR MEASUREMENTS AND AREA-BASED VOLUME ESTIMATES.** CC Schwarzwald, D Berthoud. Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Echocardiographic assessment of left ventricular (LV) size and systolic function in horses is commonly limited to subjective evaluation, measurement of LV diameter in short-axis at the chordal level, and calculation of LV fractional shortening (FS). The goal of this study was to compare the conventional unidimensional indices of LV size and function to LV volume estimates that are based on linear measurements and two-dimensional (area) measurements of the LV.

39 healthy horses (11 (4-23) y; 554 (350-660) kg) [mean (range)] and 96 horses with suspected or confirmed cardiac disease (14 (3-32) y, 550 (315-720) kg) were included in the study. Echo-cardiograms were recorded by a single observer following a standard protocol (GE Vivid 7 Dimension). The LV internal diameter was measured at end diastole (LVIDd) and at peak systole from a right-parasternal short-axis M-mode recording at the chordal level. The LV volume was estimated at end-diastole (LVIVd) and at peak systole using single-plane Simpson's model (S) and the area-length method (AL) from a right-parasternal 4-chamber view and using the bullet method (B) based on right-

parasternal long-axis and short-axis recordings. LV fractional shortening (FS) and ejection fraction (EF) were calculated. Triplicate measurements were performed blinded and in random order. LV dimensions were corrected for differences in body weight using aortic annular diameter (AAD) as an internal reference of body size. Normal reference ranges were calculated (5-95% percentile). Method comparison was performed using linear regression, Bland-Altman analyses, and weighted Kappa (κ). The level of significance was 0.05.

LVIVd was significantly related to LVIDd ($p < 0.0001$; $R^2 = 0.75$ [S], 0.75 [AL], 0.83 [B]). The % difference between volumetric methods [mean bias (limits of agreement)] was -4.8 (-8.2 to -1.5)% [S-AL], -2.9 (-23.4 to +17.5)% [S-B], and +1.9 (-18.9 to +22.7)% [AL-B]. Method agreement for classification in reduced, normal, and enlarged LV dimensions was moderate for LVIDd/AAD vs. LVIVd[S]/$AAD^3$ ($\kappa = 0.48$) and LVIVd[AL]/$AAD^3$ ($\kappa = 0.51$) and good for LVIVd[B]/$AAD^3$ ($\kappa = 0.62$). The relationship between EF and FS was significant but mostly weak ($p < 0.0001$; $R^2 = 0.15$ [S], 0.19 [AL], 0.63 [B]). The absolute difference in EF between volumetric methods was -1.1 (-2.9 to +0.7)% [S-AL], -4.7 (-14.7 to +5.3)% [S-B], and -3.6 (-13.0 to +5.9) [AL-B]. Method agreement for classification in reduced, normal, and enhanced LV systolic function was poor for FS vs. EF[S] ($\kappa = 0.18$) and EF[AL] ($\kappa = 0.08$) and moderate for FS vs. EF[B] ($\kappa = 0.53$).

Volumetric methods may be used to complement subjective evaluation and linear measurements. Best agreement was seen between conventional indices of LV size and function and volumetric estimates using the bullet method. However, further studies will be required to identify the most accurate and reliable method for assessment of LV size and systolic function in horses.

## ABSTRACT E-2

**VALIDATION OF A POINT OF CARE ULTRASOUND DEVICE FOR THE ASSESSMENT OF CARDIAC DISEASE IN THE HORSE.** AG Bevan, GD Hallowell, IM Bowen. School of Veterinary Medicine and Science. University of Nottingham. UK

Point of care (POC) ultrasound devices offer the potential advantage of portability and increased availability in equine practice due to their low cost. This study will determine the reliability of such a device (V-Scan; GE Healthcare) compared to a hospital-based (HB) device (MyLab30; Esaote) for the assessment of cardiac size, cardiac function and valvular regurgitation. The POC device lacks M-mode capability and has limited depth of imaging (24cm) so the study will also validate an alternative method for assessment of left ventricular (LV) dimensions.

Nine Thoroughbred racehorses underwent cardiac assessment on three separate occasions by one operator and on one occasion by two further operators using both the POC and HB devices. 2D images were obtained and dimensions of the aorta (Ao), pulmonary artery (PA) and left atrium (LA) were determined. LV dimensions were assessed using a standard M-mode image obtained through the left ventricle using the HB device. These measurements were also determined in systole and diastole from 2D long and short axis images of the left ventricle using the HB and, where possible, the POC device. An apically orientated left-parasternal long axis view of the LV was also used. Valvular regurgitation was identified and semi-quantitatively assessed using Color Flow Doppler. The repeatability of measurement from each device was determined both within and between operators using ANOVA and intraclass correlation. LV dimensions obtained between devices and between different methods of assessment were compared using linear regression and Bland-Altman analysis. Image quality and the presence and severity of valvular regurgitation were also compared using Fishers exact tests and calculation of Cohen's Kappa.

Although image quality was less good with the POC device, images were always of a suitable quality to enable measurements to be made. Two horses could not be manually restrained when using the HB device and their data was excluded from further analysis. Inter and intra-observer repeatability was good (ICC > 0.6) or excellent (ICC > 0.8) for both devices. There was good correlation between 2D measurements of the LA ($r^2 = 0.85$), PA ($r^2 = 0.9$) and Ao ($r^2 = 0.89$) using the two devices. There were no differences in LV measurements using the different techniques (Bias 3.4mm). Mitral regurgitation was identified in all horses using both devices, aortic and tricuspid regurgitation was identified in 6 horses using both devices. The agreement between grades of severity was good for both aortic and tricuspid regurgitation (Kappa of 0.9 and 0.6 respectively) but poor for mitral (Kappa = 0.04), although neither machine could be classed as being superior.

The POC machine provides reliable measurements of cardiac size and for the documentation of valvular regurgitation. The limited depth of imaging can be overcome allowing assessment of LV dimensions. The reduced image quality did not limit its ability to provide images that are suitable for assessment of cardiac size.

## ABSTRACT E-3

**DOPPLER AND VOLUMETRIC ECHOCARDIOGRAPHIC METHODS FOR CARDIAC OUTPUT MEASUREMENT IN STANDING ADULT HORSES.** E McConachie, MH Barton, G Rapoport, S Giguère. Departments of Large Animal Medicine and Small Animal Medicine and Surgery, University of Georgia, Athens, GA

The purpose of this study was to assess and validate various echocardiographic methods of measuring cardiac output (CO) in adult horses by comparing results to the lithium dilution method.

In eight healthy adult horses, CO was manipulated with continuous rate infusions of dobutamine and romifidine to achieve high and low CO, respectively. CO at each level (baseline, high, and low) was measured by lithium dilution and various echocardiographic methods. Images were stored as video loops for review by an individual blinded to the lithium dilution results. Limits of agreement and bias between methods were determined by Bland-Altman analysis. A two-way ANOVA was conducted to assess the effect of CO method and level of CO on bias. Significance was set at $P < 0.05$.

Lithium determinations of CO ranged between 16.6 and 63.0 L/min. There was a significant effect of method of CO measurement ($P < 0.001$) but no significant effect of CO level ($P = 0.089$) or interaction between level and method ($P = 0.607$). The absolute values of the bias of the 4 chamber area-length, Simpson, Doppler from the right ventricular outflow tract (RVOT), and bullet methods [6.5, 6.1, 5.5, 8.8 L/min, respectively] were significantly lower than that of Doppler of the left ventricular outflow tract or cubic methods [14.8, 24.3 L/min, respectively].

The 4 chamber area-length, Simpson, bullet and Doppler from the RVOT have acceptable agreement with lithium dilution and provide a non-invasive method for CO estimation in adult horses in a clinical setting.

## ABSTRACT E-4

**EFFECTS OF PRE-RACE FUROSEMIDE ADMINISTRATION ON PLASMA ELECTROLYTE CONCENTRATIONS AND ACID-BASE BALANCE IN STANDARDBREDS UNDERGOING A SIMULATED RACE PROTOCOL.** S Tinkler, L Couëtil, PD Constable. Purdue University College of Veterinary Medicine, West-Lafayette, IN

Furosemide induces a clinically significant hypochloremic, hypokalemic, hypocalcemic, metabolic alkalosis as well as marked reduction in plasma volume in sedentary horses. We hypothesized that the pre-race administration of furosemide would either augment or mitigate the profound acid-base, electrolyte, and free water changes that occur in horses during and after a simulated race.

Seven Standardbreds (2 to 6 years of age; 4 females, 3 geldings) were fed a typical training diet (DCAD $\approx$ 200 mEq/kg DM) and trained 5 times a week for 6 weeks using a high-speed treadmill. Furosemide was administered intravenously (IV) at 0, 250 mg, and 500 mg in a randomized cross-over design 4 hours

before horses underwent a simulated race protocol (SRP) on a high-speed treadmill. Jugular venous blood samples were collected via an indwelling catheter immediately before treatment and periodically up to 90 minutes after the SRP. Plasma Na, K, and Cl concentrations were determined using a Nova-4 analyzer (Nova Biomedical Waltham, MA), plasma protein concentrations were measured using refractometry and blood pH and gas tensions were determined using an ABL-5 blood gas analyzer (Radiometer Copenhagen, Denmark). Data were analyzed using repeated measures analysis of variance and $P < 0.05$ was significant.

Furosemide administration induced hypochloremia, hypokalemia, metabolic alkalosis, and alkalemia as well as a marked reduction in plasma volume by 4 hours. Pre-race furosemide administration caused a small dose-dependent decrease in plasma chloride concentrations up to 4.5 mEq/L at all time-points from fatigue to 90 minutes post SRP, as well as a dose-dependent increase in plasma total protein concentration of up to 0.7 g/dL at all time-points from fatigue to 90 minutes post SRP. Interestingly, although plasma potassium concentration peaked at 7.1±0.2 mEq/L at fatigue and then rapidly decreased to 2.8±0.2 mEq/L at 90 minutes post SRP in untreated controls, neither dose of furosemide significantly changed plasma potassium concentration during or after the SRP, compared to control. However, at fatigue, plasma potassium concentration was numerically lower in horses receiving 500 mg (6.6±0.6 mEq/L) or 250 mg (6.5±0.5 mEq/L) of furosemide IV than in untreated controls.

We conclude that the pre-race administration of furosemide (500 or 250 mg, IV) does not markedly alter the profound acid-base and electrolyte changes that occur in horses during and after a simulated race, but does have a small additive effect on the plasma volume reduction that occurs during high intensity exercise. Our results suggest that IV furosemide is unlikely to increase the incidence of cardiac arrhythmias in exercising racehorses induced by changes in plasma potassium concentration.

## ABSTRACT E-5

**ASSOCIATION BETWEEN EXERCISE-INDUCED PULMONARY HAEMORRHAGE AND DURATION OF RACING CAREER IN THOROUGHBRED RACE HORSES.** SL Sullivan, MA Jackson, GA Anderson, PS Morley[#], KW Hinchcliff. University of Melbourne, Victoria, Australia, [#]College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado, USA

Exercise-induced pulmonary hemorrhage is associated with impaired performance but the long term consequences to the health and well being of horses are unknown. We assessed the association between severity of EIPH and duration of racing career in Thoroughbred race horses. Tracheobronchoscopic examinations were performed during 2003 on 744 Thoroughbred horses within 2 hours after completing a race and the severity of exercise-induced pulmonary hemorrhage recorded (Grades 0-4). Race records of horses as of May 1st, 2011 were then reviewed to determine the duration of the horse's racing career. The association between duration of the racing career and age at enrolment, sex (male or female), and severity of EIPH were evaluated using survival analysis. All models included age and sex.

Median career durations were 18.7, 17.2, 16.3, 19.6 and 5.9 months for the 332, 273, 101, 25 and 13 horses with EIPH grades 0, 1, 2, 3 and 4 respectively. The hazard ratio for EIPH grade $\geq$ 1 versus 0 was 1.07 (95%CI 0.9–1.2, $P = 0.37$) and for EIPH grade $\geq$ 2 versus $\leq$ 1 was 1.07 (0.9–1.3, $P = 0.45$). Males had a significantly increased career duration ($P < 0.001$) and increasing age reduced the career duration ($P < 0.001$). Hazard ratios for EIPH 1, 2, 3, or 4 vs 0 were 1.05 (95%CI 0.9-1.2,$P = 0.53$), 1.04 (0.8-1.3,$P = 0.74$), 1.09 (0.7-1.6, $P = 0.67$) and 2.06 (1.2-3.6, $P = 0.011$), respectively. Only the most severe EIPH was associated with reduced duration of racing career. This could be attributable to racing regulations which mandate a three month withdrawal from racing for horses with EIPH and epistaxis and disqualification from racing after a second episode of EIPH with epistaxis.

## ABSTRACT E-6

**HYPERBARIC OXYGEN THERAPY DOES NOT ALTER mRNA INFLAMMATORY GENES EXPRESSION IN HEALTHY HORSES.** M Looijen[1], R Dardari[1], R Leguillette[1,2]. [1]University of Calgary, Faculty of Veterinary Medicine, Calgary, AB, Canada, [2]Moore Equine Veterinary Centre, Calgary, AB, Canada

Hyperbaric oxygen therapy (HBOT) induces an oxidative stress over time that may alter the mRNA expression of inflammatory cytokines and housekeeping genes in lung cells of horses. Identifying stable reference genes is necessary to obtain reliable quantitative PCR (QPCR) results. Therefore, the objectives of this study were 1) to identify the most stable reference genes in the bronchoalveolar lavage fluid (BALF) cells of horses treated with HBOT and 2) to evaluate if Th1, Th2 or Th17 inflammatory cytokines expression was increased in the BALF of the horses treated with HBOT.

Eight horses were used in a randomized controlled cross-over design. Treated horses were exposed to 100% oxygen at 3 ATA for 20 minutes for 10 days whereas the chamber was not pressurized for control horses. The mRNA expression of IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p35, IFN-γ, TNF-α and Eotaxin-2 was measured by QPCR in BALF sampled pre- and post-HBOT treatment. Genes' expression was measured by QPCR after efficiency correction using REST software analysis. The expression stability of four candidate reference genes (GAPDH, HPRT, SDHA and RPL-32) was determined using Normfinder and Genorm$^{plus}$.

GAPDH was found to be the most stable reference gene using both Genorm$^{plus}$ and Normfinder. The number of reference genes used for optimal normalisation included GAPDH and HPRT. The mRNA expression of cytokines was not significantly up- or down- regulated with HBOT, however there was a significant down-regulation for Eotaxin-2 between the HBOT and the control group after HBOT.

## ABSTRACT E-7

**TEMPORAL VIRAL LOAD IN SEVEN HORSES WITH NATURALLY OCCURRING EQUINE HERPESVIRUS-1 MYELOENCEPHALOPATHY.** KE Estell, D Dawson, KG Magdesian, S Mapes, E Swain, N Pusterla. School of Veterinary Medicine, University of California, Davis, CA

The pathogenesis of equine herpesvirus-1 myeloencephalopathy (EHM) is poorly understood and is likely a result of viral, host, and environmental factors. It has been hypothesized that the magnitude of viremia is a central factor in the development and severity of EHM; however, temporal viral load has never been reported in a naturally occurring cases. Additionally, prognosis for grade 4/5 neurologic horses has not been reported. The purpose of this case series is to report the clinical signs, treatment, and temporal viral load in 7 horses with naturally occurring EHM and examine these factors' association with survival.

Seven horses with EHM presented to the UC Davis VMTH between 5/13/11 and 9/20/11 and were associated with 3 separate outbreaks. All horses were graded on presentation using the neurologic grading scale. Out of 7 horses, 1 horse was grade 5/5, 3 horses were grade 4/5 and 3 horses were grade 3/5. All horses received valacyclovir, flunixin meglumine, DMSO, vitamin E, and supportive care. Urinary incontinence was present in all horses and was treated with bladder catheterization and antimicrobials for the duration of urinary catheterization. All horses presenting with > grade 3/5 neurologic signs received dexamethasone and were maintained in the Anderson sling support device.

All 7 horses were infected with the $D_{752}$ genotype of EHV-1. Quantitative PCR was performed on nasal secretions and whole blood collected at admission and every 24 hours until horses were negative for >2 days, died, or were euthanized. Viral load was quantified and expressed as *glycoprotein B* (*gB*) gene copies per million cells. Peak viral load in nasal secretion of horses which survived ranged from 6,187-281,102 *gB* gene copies per million cells (median 5,179); peak viral load in blood was 143-4,338 *gB*

gene copies per million cells (median 3,146). The 2 non-surviving horses presented with grade 3/5 neurologic signs and progressed rapidly to recumbency with signs of encephalopathy. Peak viral load was considerably higher in non-survivors with levels in nasal secretions of 192,019,861- 221,904,282 *gB* gene copies per million cells (median 192,019,861) and levels in blood of 20,467-102,491 *gB* gene copies per million cells (median 47,359). All horses that survived showed cessation of viral shedding in nasal secretions by day 5 of hospitalization.

Overall mortality rate was 28.6% (2/7). All horses that did not develop encephalopathy survived. We hypothesize that the magnitude of viral load is associated with outcome. Non-survivors had viral loads that were 1000-fold higher in nasal secretions and 10-fold higher in blood than non-survivors. There was no relationship between severity of clinical signs at presentation with survival, emphasizing the value of supportive care including management in a sling if necessary.

## ABSTRACT E-8

## INVESTIGATION OF THE ROLE OF MULES DURING AN OUTBREAK OF EQUINE HERPESVIRUS-1 MYELOENCEPHALOPATHY IN CALIFORNIA.

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Although all breeds of horses are susceptible to the neurologic form of EHV-1 infection, the authors are unaware of reports of neurologic EHV-1 clinically affecting donkeys and mules. However, donkeys and mules have shown seroconversion indicating infection with EHV-1 while in contact with affected horses during outbreaks. The goal of this study was to investigate the role of mules as possible silent shedders during an outbreak of EHM.

In early September of 2011, an EHV-1 outbreak with several neurologic horses occurred at a packing station located in the eastern Sierra of California. The packing station was visited with the goal to assess the clinical status and exposure rate of the resident horses and mules. Using appropriate biosecurity measures, a physical evaluation was performed followed by the collection of whole blood and nasal secretions for the quantitative PCR and serological detection of EHV-1.

A total of 141 horses and mules were evaluated during the visit. There were 82 horses and 59 mules ranging in age from 3 to 30 years (median 12 years). On the day of the visit a total of 5 additional horses showed neurological deficits. Fifty-six horses or mules (39.7%) tested PCR positive for the EHV-1 *gB* and the *ORF 30* ($D_{752}$) gene in blood (13 animals), nasal secretions (24) or both (19). The viral load determined by PCR in blood and nasal secretions ranged from 27 to 8,542 *gB* gene copies/million cells (median 443 *gB* gene copies/million cells) and from 66 to 3.5 x $10^6$*gB* gene copies/million cells (median 2,485 *gB* gene copies/million cells), respectively. In an attempt to determine statistical differences in viral loads, the PCR positive horses and mules were grouped into asymptomatic horses (27 animals), asymptomatic mules (24) and horses with neurological signs (6). The viral loads in blood were significantly different between the three groups (Wilcoxon-Mann-Whitney tests; $P < 0.05$) with asymptomatic horses having the lowest viral loads and neurologic horses showing the highest viral loads. For nasal secretions, asymptomatic mules had significantly higher viral loads in nasal secretions ($P < 0.05$) compared to asymptomatic horses. A total of 33/141 (23.4%) horses and mules tested seropositive for EHV-1. Based on combined molecular and serological testing 48/82 (58.5%) horses and 32/59 (54.2%) mules had evidence of EHV-1 exposure.

The results of this study showed that equal percentages of horses and mules became exposed to EHV-1 during an EHM outbreak; however, neurological disease was only reported in horses. Mules seem to be protected from the development of neurological signs by means yet to be determined. However, the molecular and serological results support the role of mules as silent shedders, highlighting the need to institute appropriate biosecurity protocols when horses and mules are comingled.

## ABSTRACT E-9

## EQUINE SALMONELLOSIS. THE USE OF AN ENHANCED RAPID TEST SYSTEM (REVEAL®2.0 SALMONELLA TEST SYSTEM) FOR EARLY DETECTION OF *SALMONELLA* IN FECES AND ENVIRONMENTAL SAMPLES.

N Slovis[4], J Elam. Hagyard Equine Medical Institute, Lexington, KY

In veterinary medicine, a rapid, inexpensive and accurate diagnosis of horses infected with Salmonella spp. is important, so that necessary control and preventative measures can be implemented to reduce the risk of disease transmission to other horses or personnel. Conventional diagnosis of Salmonella spp. is conducted by testing fecal samples using bacteriologic procedures in the laboratory. However, it can take up to 3 to 5 days to obtain laboratory results. Several studies have developed PCR tests for detection of Salmonella spp. in fecal and environmental samples but there is evidence of an increased frequency of Salmonella-PCR positive results in horses without clinical signs of salmonellosis that test negative to Salmonella spp by culture on multiple fecal samples, perhaps due to the use of primers targeting a non-specific Salmonella spp gene fragment that may cross-react with other enteric or non-enteric organisms. The objective of the study is to assess the diagnostic accuracy of an enhanced rapid test system *(Reveal ® 2.0 Salmonella test system)* for the detection of Salmonella spp. in fecal and environmental samples, compared to bacteriological culture identification.

The samples were initially plated to a hektoen agar and inoculated in a selenite broth. After 24 hours, the hektoen agar was examined for black or green colonies. The selenite broth was subbed to a hektoen agar, incubated for 24 hours and then examined for black or green colonies. The Reveal ®test would be performed on any black or green colonies found on either hektoen agar.

Our lab has performed 810 Reveal® tests on suspicious cultures from January 16, 2010 to December 31, 2011. There were 150 Reveal® positive samples and 660 negative samples. There was 100% correlation (100% sensitivity and 100% specificity) of the Reveal® test results to the BBL Crystal identification results.

The Reveal ®test has been shown in our lab to save 24 hour to 48 hours in notifying the veterinarian of a positive Salmonella culture.

Further studies are warranted with the Reveal ® test system on Selenite broths. If similar correlations are noted with the broths then *Salmonella* results may be able to be obtained in a 24 hour turnaround time.

## ABSTRACT E-10

## ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* ISOLATES OBTAINED FROM HORSES IN THE NORTHEASTERN UNITED STATES (2001-2010).

GA Perkins[1], KJ Cummings[1,2], SM Khatibzadah[1], J Stiler[1], LD Warnick[1], C Altier[1]. [1]Cornell University, Ithaca, NY, [2]Texas A&M University, College Station, TX

The objectives of this study were to describe the antimicrobial resistance status of equine *Salmonella* isolates recently cultured in the northeastern U.S. and to identify trends in resistance over time.

Data were collected retrospectively for all equine *Salmonella* isolates with antimicrobial minimum inhibitory concentrations (MICs) that were isolated at the Cornell University Animal Health Diagnostic Center from January 1, 2001 to December 31, 2010. MICs of isolates were determined using the microbroth dilution method (TREK Sensititre).

A total of 459 equine *Salmonella* isolates with antimicrobial susceptibility testing were retrieved (median of 45 isolates per year; range 20- 83). The majority of isolates were from clinical submissions (feces) either from regional veterinarians or the Cornell University Hospital for Animals. Most isolates (98.4-100%) were susceptible to amikacin, enrofloxacin and imipenem. Individual Cochran-Armitage tests showed a significantly decreasing trend over time ($p \leq 0.005$) in prevalence of resistance to amoxicillin/clavulanic acid (AUG), ampicillin (AMP), cefazolin, cefoxitin (FOX), ceftiofur (TIO), chloramphenicol (CHL) and tetracycline (TET). Increasing resistance was not found for any of the 16 antimicrobials tested. There were 380 isolates that

tested nine available National Antimicrobial Resistance Monitoring System panel drugs. The most common resistance patterns were pan-susceptible (38.2%), AUG-AMP-FOX-TIO-CHL-TET (16.6%), AUG-AMP-FOX-TIO-CHL-TET-Timethoprim/sulfamethoxazole (SXT) (12.4%), AUG-AMP-FOX-TIO-CHL-TET-SXT-Gentamicin (GEN) (11.3%), and AMP-TIO-CHL-TET-SXT-GEN (7.6%).

Judicious use of fluoroquinolones would be appropriate for the treatment of severe salmonellosis in horses in the northeastern U.S. These results suggest that the emergence and dissemination of resistant *Salmonella* isolates is complex and may not be related to overall antimicrobial use.

**ABSTRACT E-11**

**ROLE OF INTRAOCULAR BACTERIAL INFECTIONS IN HORSES WITH RECURRENT UVEITIS IN LOUISIANA.** F Polle, RT Carter. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA

To investigate the role of intraocular bacterial infections in horses with Equine Recurrent Uveitis (ERU), eyes were harvested from donated horses with a history and ocular findings consistent with chronic ERU. Additionally, eyes were collected from horses with normal ophthalmic examinations as a control group. Blood samples were obtained for *Leptospira* serology using microscopic agglutination test (MAT). Following euthanasia, aqueous and vitreous humor samples were aseptically obtained and submitted for aerobic culture and *Leptospira* culture, PCR and MAT.

Twenty-one control horses (41 eyes) and 15 ERU horses (27 eyes) were available for study. Serology results were available for 35/36 horses: 16/21 control and 13/14 affected horses were positive for at least one serovar; Bratislava was the most common serovar for both groups. *Bacillus sp*. was cultured from one control eye; *Streptococcus sp*. (n = 1) and *Leptospira* (n = 1) from two eyes with ERU. PCR results were positive in 10/27 (37%) eyes from horses diagnosed with uveitis; no control horses were positive by PCR. MAT was positive for 20/26 tested eyes (77%) with uveitis and 1/41 (2.4%) of normal eyes. PCR and MAT results were in agreement for 55/67 (83%) of eyes sampled. Horses with ERU had a high prevalence of *Leptospira* infection based on PCR and MAT results from intraocular fluids compared to controls. *Leptospira* infection should be considered as a cause of ERU in Louisiana. The diagnosis of these intraocular infections was not aided by serology and required specific, invasive sampling of the ocular fluids.

**ABSTRACT E-12**

**GENETIC DETERMINANTS OF MELANOMA SUSCEPTIBILITY IN GRAY HORSES.** R Teixeira[1], M McCue[1], S Anderson[2], D Sigler[3], B Buchanan[4], B Coleman[5], J Mickelson[2]. [1]Veterinary Population Medicine, [2]Veterinary Biomedical Science, University of Minnesota. St Paul, MN, [3]Department of Animal Science, Texas A & M University, College Station, TX, [4]Navasota, TX, [5]Department of Animal Science, University of Kentucky, Lexington, KY

A correlation between the gray coat color and melanoma development has long been recognized with up to 80% of gray horses older than 15 years developing melanoma. Both graying and melanoma formation have been linked to a duplication in the Syntaxin 17 (*STX17*) gene, with the *STX17* genotype affecting melanoma grade and severity in the Lipizzaner breed. An *ASIP* mutation, responsible for bay/black coat color modifies melanoma risk in gray horses, with increased melanoma severity potentially due to an increase in MC1R pathway signaling. We have identified a population of gray Quarter Horses (QH) that appear to have a *decreased* melanoma risk. Dermal melanoma is very low to non-existent is some pedigrees, suggesting there are other genetic influences in addition to gray coat color that play a role in melanoma development. The reason for the decreased incidence of melanoma in these horses is unclear, however based on the importance of increased MC1R signaling in melanoma susceptibility with the *ASIP* mutation; we believe that the cellular mechanism responsible for decreased melanoma susceptibility may also involve this pathway. The objective of this study was to determine if the decreased incidence of melanoma in gray QH was related to decreased MC1R signaling resulting from the *MC1R* chestnut coat color allele

318 gray QH (Age: 1 to 33 years old; Mean age: 9 years old) were phenotyped for melanoma (scale 0 to 4; 0 = no melanomas, 4 = ulcerated/metastatic melanomas). In this cohort, mean melanoma grade was 0.38 and the incidence was 16.9%, compared to incidences of 50% and 31.4% in the Lipizzaner and Camargue breeds, respectively. Further, melanoma incidence in QH older than 15 years was lower (55.8 %) than published incidences in Lipizzaner (75%) or Camargue (68%) horses older than 15 years. 291 horses were genotyped for *MC1R*, *ASIP* and *STX17*. Univariate and multivariate regression analyses were performed to determine the relationships between genotype and age with melanoma incidence and grade. Age was associated with melanoma incidence and severity in both univariate and multivariate analyses ($p = 2.0 \times 10^{-16}$). However, no effect of *MC1R, ASIP* or *STX17* genotypes was found in either analysis. Prior studies have demonstrated that *STX17* genotype is the major determinant of melanoma grade, with homozygous horses being at a greater risk of developing melanomas. In our cohort, only 15 (5%) of gray horses were homozygous, resulting in poor statistical power to demonstrate the effect of *STX17* genotype. Previous studies have also shown that the *ASIP* mutation increases melanoma risk. *ASIP* allele frequency in our study was low (8.7%), resulting in poor statistical power to detect an effect of *ASIP* genotype. Our findings confirm a low incidence and severity of melanoma and a high frequency of the MC1R mutation (58.7%) in our gray QH population. Further work is needed to determine if the decreased incidence and severity of melanoma in gray QHs is due to infrequent *STX17* homozygosity and/or low *ASIP* frequency, or due to other genetic factors.

**ABSTRACT E-13**

**EFFECT OF BODY POSITION ON ABDOMINAL PRESSURES IN ADULT HORSES.** VHL Scott, JL Williams, MC Mudge, SDA Hurcombe. The Ohio State University, Columbus, OH

Accurate interpretation of intra-abdominal pressure (IAP) is essential in documenting intra-abdominal hypertension; a significant cause of morbidity in critically ill humans, which has also been described in horses with colic. Body position is known to alter intra-abdominal pressure (IAP) measurements in people, leading to the development of standardized acquisition methodologies to minimize variability. To date, such information is lacking in equine studies. The purpose of this study was to investigate the effect of body position on direct IAP (dIAP) and abdominal perfusion pressure (APP) in normal adult horses.

Nine healthy adult horses were included and a standardized total intravenous anesthetic protocol was used to facilitate patient positioning. dIAP via abdominal cannulation and electronic sphygmomanometry was measured from the left flank (LFl), right flank (RFl) and ventral (V) abdomen with horses randomly positioned into left lateral (LLR), right lateral (RLR) and dorsal recumbencies (DR). Direct mean arterial blood pressure (MAP) was obtained concurrently to calculate APP (MAP - IAP). Horses were placed in each recumbency twice, repeatability assessed and mean values of IAP/MAP/APP measured in triplicate were used for statistical analysis. Differences in IAP/MAP/APP at specific sites for each body position were assessed by paired t-tests or repeated measures ANOVA. $P < 0.05$ was significant.

Ventrum dIAP was significantly lower with horses in DR (-7 mmHg) compared to values obtained in LLR or RLR (11 mmHg

and 13 mmHg respectively); $P < 0.0001$. Ventrum APP was not different with horses in all 3 recumbencies (DR = 76 mmHg, LLR = 80 mmHg, RLR = 72 mmHg). MAP was significantly lower when horses were positioned in DR (69 mmHg) compared to LLR or RLR (83 mmHg and 94 mmHg respectively); $P < 0.0001$. LFl dIAP was significantly lower (-5 mmHg) and APP significantly higher (100 mmHg) with horses in RLR compared to DR (15 mmHg and 54 mmHg respectively); $P < 0.0001$ for both dIAP and APP. RFl dIAP was significantly lower (-8 mmHg; $P < 0.001$) and APP significantly higher (91 mmHg; $P = 0.002$) with horses in LLR compared to DR (15 mmHg and 55 mmHg respectively)

Body position directly affects intra-abdominal and hemodynamic pressures. These effects should be considered when evaluating abdominal pressure profiles and visceral perfusion in horses.

## ABSTRACT E-14

## VOLUME OF DISTENDED SMALL INTESTINE AND IONIC COMPSITION OF GASTRIC REFLUX AND SMALL INTESTINAL FLUID IN HORSES.

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Distension of the small intestine (SI) is a common finding in horses with obstructive disorders, duodenitis-jejunitis, and postoperative ileus. Removal of fluid accumulated in the stomach, largely SI secretions, via a nasogastric tube is an essential part of management of affected horses and can result in substantial fluid and electrolyte losses. There is little published data on the quantitative amount of water and electrolyte losses that may be lost through nasogastric reflux or with surgical resection of devitalized and distended SI. The purpose of this study was to determine the volume of water that would cause distension of the duodenum and various lengths of jejunum to 6-7 cm diameter as well as the concentration of electrolytes in normal SI ingesta, nasogastric reflux fluid from horses with colic, or fluid in distended SI collected from horses undergoing surgical resection of devitalized SI. First, various lengths (7 to 45 feet) of healthy SI were collected immediately following euthanasia from 15 horses that did not have colic signs and warm (~30°C) water was slowly infused to distend the SI to a diameter of 6-7 cm. Water was then drained from the SI and volumes were measured (± 0.5 L) and correlated to the length of the SI that was distended. Second, $Na^+$, $K^+$, and $Cl^-$ concentrations were measured in 23 intestinal fluid samples (10 SI ingesta samples collected from healthy horses, 11 initial spontaneous gastric reflux samples, and 2 SI fluid samples collected during surgery). Correlation analysis revealed that healthy SI is able to hold 0.81 L/foot of warm water ($r = 0.92$, $p < 0.01$, $n = 15$). Concentrations of $Na^+$, $K^+$, and $Cl^-$ are detailed in the table:

| Sample | $Na^+$ mmol/L mean ± SD (range) | $K^+$ mmol/L mean ± SD (range) | $Cl^-$ mmol/L mean ± SD (range) |
|---|---|---|---|
| SI ingesta, n = 10 | 100 ± 6 (21-109)[a] | 22 ± 5 (11-31)[a] | 66 ± 30 (21-109)[a] |
| nasogastric reflux, n = 11 | 77 ± 16 (44-102)[b] | 16 ± 11 (4-40)[a] | 96 ± 25 (60-142)[b] |
| distended SI fluid, n = 2 | 116 ± 11 (108-124)[a] | 12 ± 5 (8-15)[a] | 43 ± 27 (24-62)[a] |

Concentrations of $Na^+$ were lower ($p < 0.01$) and $Cl^-$ greater ($p < 0.03$) in nasogastric reflux fluid than in normal SI ingesta or fluid collected from distended SI at surgery. These data can be used to estimate the magnitude of water and electrolyte losses that may occur with SI disorders in order to implement appropriate fluid therapy plans for affected horses.

## ABSTRACT E-15

## PREVALENCE AND RISK FACTORS FOR ULCERATION OF THE GASTRIC GLANDULAR MUCOSA IN THOROUGHBRED RACEHORSES IN TRAINING IN THE UK AND AUSTRALIA.

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Equine gastric ulcer syndrome is a common clinical condition which can cause poor performance in racing Thoroughbreds worldwide. Although squamous gastric ulceration has been widely studied, evaluation of the prevalence and risk factors for glandular ulcers has not. The study was performed to determine the prevalence of ulceration in the glandular and pyloric regions in Thoroughbred racehorses in training in the UK and Australia (AUS) and identify associated risk factors. 113 horses from 3 training yards in the UK and 5 training yards in AUS were studied. Gastroscopy was performed on each horse after a period of starvation and under sedation. All squamous, glandular and pyloric regions were assessed for evidence of ulcers. Ulcers were scored 0 to 4 depending on severity (EGUS Council System). A comprehensive questionnaire which included signalment, detailed information regarding training, feeding, general management practice, racing results, recent medication administration and any previous veterinary history was completed by the trainer for each horse. Univariable logistic regression analyses and then multivariable mixed effect logistic regression models were built in a stepwise fashion to identify risk factors for glandular and squamous gastric ulceration. Significance was assumed as $p < 0.05$.

The overall prevalence of glandular ulceration was 50.4%, squamous ulceration was 73.5% and horses with both squamous and glandular ulceration was 39.8%. There was a significant relationship between glandular and squamous scores ($p < 0.001$). Horses in AUS had a higher prevalence of glandular ulceration than those in the UK ($p < 0.001$). The prevalence of squamous ulceration was not different between the UK and AUS horses ($p = 0.32$). Risk factors for glandular ulceration were gender ($p < 0.01$; colts were less likely to have ulcers than mares or geldings), trainer ($p < 0.001$), no grass turnout ($p < 0.006$), horses in direct contact with each other ($p < 0.001$), horses not fed haylage ($p < 0.002$), horses fed unprocessed grain ($p < 0.003$), horses that were infrequently fed a complete diet ($p < 0.001$), horses that underwent fast exercise on fewer days of the week ($p < 0.001$) and that went swimming ($p < 0.001$). Fewer risk factors were identified for squamous ulceration. Increased squamous ulcer prevalence was noted in horses in work for a longer period of time ($p < 0.048$; this was not observed for glandular ulceration ($p = 0.54$)). A lower prevalence of squamous ulceration was also noted in horses aggressive towards humans ($p < 0.01$). In conclusion, many risk factors were identified pertaining to glandular ulceration; these were different to those for squamous ulceration. The strong association between the concurrent presence of squamous and glandular ulcers has not previously been reported nor have any the regional differences in glandular ulceration prevalence.

## ABSTRACT E-16

## REGULATION OF HYPOXIA INDUCIBLE FACTOR-1A AND RELATED GENES IN THE EQUINE KERATINOCYTE AND DIGITAL LAMINAE IN EXPERIMENTAL MODELS OF EQUINE LAMINITIS.

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Hypoxia inducible factor-1 alpha (HIF-1A) is a central protein in the cellular response to both hypoxia and inflammatory signaling (i.e. NF B activation via TLR4 signaling). Increased cellular HIF-1A concentrations, occurring most commonly from either increased transcription or decreased proteosomal degradation, lead to the induction of HIF-1A-responsive genes. Hypoxia alone can induce a HIF-1A-mediated increase in genes involved in energy regulation (e.g. glucose transporter 1 [GLUT1], phosphoglycerokinase-1[PGK1]) whereas NFkB activation is reported to induce a HIF-1A-mediated increase in inflammatory genes (e.g. nitric oxide synthase-2 [NOS2] and cyclooxygenase-2 [COX-2]).

Because of the implication of hypoxic and inflammatory events in the pathogenesis of equine laminitis and the specific importance of the basal epidermal keratinocyte in maintaining lamellar function, this study sought to determine the regulation of HIF-1A and HIF-1A-responsive genes in both equine keratinocytes and the equine digital lamina as a whole. Immunochemistry (HIF-1A), and real time-quantitative PCR (HIF-1A, GLUT1, PGK1, NOS2, COX-2) were used to assess gene/protein regulation in 1) cultured equine keratinocytes (skin) exposed to LPS at either normoxic or hypoxic ($3\%O_2$) conditions and 2) laminar sections from control animals (CON) and animals at developmental (DEV) and Obel grade 1 lameness (OG1) time points in the carbohydrate and black walnut extract models of laminitis. The laminar keratinocyte was the primary cell type positive for HIF-1A via tissue immunofluorescence, indicating that, similar to skin keratinocytes, the laminar keratinocyte exists in a hypoxic environment. Hypoxic cultured keratinocytes underwent marked increases in cellular concentrations of HIF-1A protein with no change in mRNA concentration. Unlike that reported in many cell types, LPS exposure did not result in increased HIF-1A concentrations in normoxic cultured keratinocytes. Hypoxia alone resulted in increased mRNA concentrations ($p < 0.05$) of GLUT1 only; however, exposure of hypoxic cells to LPS resulted in increased mRNA concentrations ($p < 0.05$) of COX-2 and NOS2. Laminar HIF-1A protein concentrations were only increased in the CHO model at the OG1 time point (vs CON, $p < 0.05$); increased laminar mRNA concentrations were present at DEV and OG1 time points for COX-2 and NOS2 ($p < 0.05$, no changes occurred in HIF-1A, VEGF, PGK1, or GLUT1 mRNA concentrations). In conclusion, these results indicate that HIF-1A is normally present in the laminar keratinocyte due to the cell constantly being in a slightly hypoxic state, and that increases in HIF-1A and in NF B-responsive HIF-1A related genes (COX-2 and NOS2) in affected laminae in models of laminitis are likely due to a TLR-related induction of NF B signaling resulting in a synergistic increase in inflammatory genes which are responsive to both HIF-1A and NF B signaling.

## ABSTRACT E-17
## LAMINAR INFLAMMATORY EVENTS IN LEAN AND OBESE PONIES SUBJECTED TO HIGH CARBOHYDRATE FEEDING: IMPLICATIONS FOR PASTURE-ASSOCIATED LAMINITIS.

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A robust laminar inflammatory response, including leukocyte infiltration and inflammatory mediator expression, has been well-characterized in laminitis induced by black walnut extract or enteral carbohydrate overload. Although inflammation has been proposed to also play a role in laminitis associated with equine metabolic syndrome, it has not been critically evaluated. The purpose of this study was to characterize the expression of inflammatory genes and leukocyte infiltration in digital laminar tissue of ponies subjected to a dietary carbohydrate challenge designed to mimic abrupt exposure to pasture rich in nonstructural carbohydrate (NSC). Following 4 weeks of conditioning consisting of a diet of hay chop (NSC ~6% on a DM basis), mixed-breed ponies (body weight 270.9 +/- 74.4 kg) were assigned to groups based on body condition scoring (lean vs. obese). Ponies either remained on the conditioning diet (CON diet; n = 5 obese, n = 5 lean) or received the same diet supplemented with sweet feed and oligofructose (CHO diet [~42% NSC]; n = 6 obese, n = 6 lean) for a period of 7 days. At the end of the feeding protocol, dorsal digital laminar tissue samples were collected immediately following euthanasia; samples were formalin-fixed or snap frozen. Laminar immunohistochemistry was performed for CD163 and MAC387/calprotectin using commercially-available antibodies; the number of immunopositive cells was quantified at 40x magnification (n = 10 fields) for each section by a blinded observer. Real-time-quantitative PCR was used to assess laminar mRNA concentrations of pro-inflammatory cytokine/chemokine genes (TNFα, IL-1β, IL-6, IL-8, MCP-1, MCP-2) and COX-2. There was no effect of diet or body condition on the number of laminar CD163(+) or MAC387(+) cells ($p > 0.05$); very few laminar MAC387+ cells were present in any group. There was no difference in the laminar mRNA concentrations of TNFα ($p = 0.92$), IL-1β ($p = 0.18$), IL-6 ($p = 0.28$), IL-8 ($p = 0.21$), MCP-1 ($p = 0.25$), or MCP-2 ($p = 0.67$); however, laminar mRNA concentrations of COX-2 ($p = 0.01$) were increased in ponies fed a high-CHO diet (vs. CON diet). These results suggest that the primary inflammatory events occurring in the marked inflammatory response reported in sepsis models of laminitis, leukocyte infiltration and proinflammatory cytokine/chemokine expression, are not central events in EMS-associated laminitis. As laminar COX-2 expression has been primarily localized to vascular wall components (endothelium and smooth muscle) and laminar keratinocytes in the normal and laminitic equid in previous reports, the increased laminar mRNA concentrations of COX-2 in this study may reflect laminar epithelial dysfunction and/or vascular pathology in the affected laminae. Ongoing immunolocalization studies will further clarify the cellular origin of the increased COX-2 expression.

## ABSTRACT E-18
## ABSENCE OF OXIDATIVE STRESS IN THE SKELETAL MUSCLE OF OBESE HORSES.

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Obesity in people is associated with insulin resistance. In horses, obesity is associated with insulin resistance and the development of laminitis. In people, obesity is associated with increased cellular reactive oxygen species production and local (tissue) and systemic oxidative stress. Increased ROS may interfere with insulin signaling, resulting in insulin resistance. However, measurements of systemic markers of oxidative stress in obese horses have not demonstrated alterations in redox status. Our objective was to determine the relationship between obesity and oxidative stress in skeletal muscle of horses. We hypothesized that obese horses have increased oxidative stress in skeletal muscle compared with non-obese horses, as demonstrated by an increase in thiobarbituric acid reactive substances (TBARS) and compensatory increase in antioxidant gene expression.

Thirteen light breed horses, 2-24 years of age with a body condition score (BCS) ranging from 4-9 were included. Plasma insulin concentrations were quantified via ELISA. RNA was isolated from biopsy samples of the semi-membranosus muscle for analysis of gene expression. Relative expression of the antioxidant genes, catalase, glutathione synthase, glutathione reductase, glutathione peroxidase, periredoxin, and manganese superoxide dismutase was determined in skeletal muscle by quantitative polymerase chain reaction (qPCR). TBARS were quantified in plasma and muscle using a commercially available kit. Non-normal data was log transformed for analysis. A Pearson's coefficient of correlation was calculated for body condition score, insulin concentration, gene expression and TBARS. Muscle TBARS were decreased in obese ($BCS > 7$; $n = 7$) compared to non-obese ($BCS \leq 5$; $n = 6$) horses (0.44±0.08 versus 1.41 ±0.26 nmol/mg). Body condition score was negatively correlated with tissue TBARS ($r = -0.56$) but not plasma TBARS ($r = -0.07$). Plasma and muscle TBARS were not correlated ($r = 0.12$). There was a negative correlation between tissue TBARS and plasma insulin (-0.56) which approached significance ($p = 0.06$). Antioxidant gene expression was not correlated with body condition score or insulin concentration.

Obese horses demonstrated a decrease in oxidative stress within skeletal muscle, which was not in support of our hypothesis. These findings indicate that obesity in horses, unlike in people, is not associated with increased reactive oxygen species production. Further investigation into the cellular mechanism of insulin resistance in the horse is warranted.

**ABSTRACT E-19**

**THE EFFECTS OF EXPERIMENTALLY INDUCED HYPERGLYCEMIA AND ENDOTOXEMIA ON COAGULATION PARAMETERS IN HEALTHY ADULT HORSES.** KF McGovern[1], KM Lascola[1], SA Smith[2], PA Wilkins[1], SC Clark-Price[1], JH Foreman[1]. [1]College of Veterinary Medicine, Urbana, IL, [2]College of Medicine, Urbana, IL

Hyperglycemia and endotoxemia have been independently associated with equine coagulation abnormalities. Experimental studies suggest a greater impact of hyperglycemia on coagulation abnormalities with concurrent induction of endotoxemia. The study purpose was to compare coagulation parameters in healthy adult horses administered LPS with and without concurrent induction of hyperglycemia.

Hyperglycemia (180-240 mg/dL) was maintained for 6 hours in 6 healthy adult horses (Group 1) using 140mg/kg IV bolus followed by 20% dextrose constant rate infusion (CRI). A similar volume of saline was administered to a further 6 adult horses (Group 2). LPS (20ng/kg) was administered to each horse. TNFα, white blood cell count, platelet count, fibrinogen concentration, prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin anti-thrombin complex level (TAT) were determined at baseline, 1,1.5,2,3,4,6 and 22h. Thromboelastometry (TEM) parameters were determined at baseline, 3,6 and 22h. Repeated Measure ANOVA and the Friedman Rank Sum test were used to determine significant differences over time.

TNFα increased ($p < 0.001$) in both groups. PT increased at 6 hours in Group 1 ($p < 0.001$). For TEM, alpha angle (In-TEM $p = 0.02$; Ex-TEM $p = 0.006$) and maximal clot firmness (Ex-TEM $p = 0.009$) decreased, and clot formation time (In-TEM $p = 0.003$; Ex-TEM $p = 0.001$) increased at 3 hours in group 2. Increases in TAT were identified in both groups but were not significant.

Alterations in coagulation parameters were identified for both groups in this study. Identified changes in markers of coagulation differed between groups. These results suggest that hypocoagulation induced by exogenous endotoxin is not worsened, but rather ameliorated, by concurrent hyperglycemia.

**ABSTRACT E-20**

**EVALUATION OF HYPERCOAGULABILTY IN GERIATRIC HORSES WITH/WITHOUT PITUITARY PARS INTERMEDIA DYSFUNCTION.** J Mahon[1], MRParadis[1], DM cFarlane[2], A Sanchez[3], LL Price[4], E Rozanski[1], D Meola[1], AM deLaforcade[1]. [1]Tufts Cummings School of Veterinary Medicine, North Grafton, MA, [2]Oklahoma State University, Stillwater, OK, [3]Tufts University Ambulatory Clinic, Woodstock, CT and 4-Tufts Medical School, Boston, MA

Pituitary Pars Intermedia Dysfunction (PPID) is the most common endocrine disorder in geriatric horses, and shares the characteristic of increased ACTH with human and canine pituitary dependent hyperadrenocorticism (PDH). PDH and aging (in humans) has been associated with evidence of systemic hypercoagulability. The goal of this study was to determine if coagulation status differed between normal healthy horses, and geriatric horses with and without PPID.

Healthy young and geriatric horses (> 20 years of age) and PPID geriatric horses were recruited between May 15th and June 15th in 2010 and 2011. Horses were categorized as PPID by the presence of hirsuitism, and if ACTH > 50 pg/mL and α-MSH > 35 pmol/dL. Horses were classified as normal based upon phenotype and an ACTH < 35 pg/ml and α-MSH of < 20 pmol/dl. Kaolin-activated thromboelastography (TEG), prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen (Fib C) were performed on all horses. T-tests were used to compare normally distributed data and Mann-Whitney for non-normally distributed data, with a $p < 0.05$ considered significant.

Forty-four horses were screened for hirsuitism, and ACTH and MSH levels. 12 were categorized as normal young horses, 8 as normal geriatric horses, and 13 as geriatric PPID horses. Eleven horses had equivocal results and were not further evaluated. There were no significant differences in any TEG variable, fibrinogen or aPTT between young and geriatric horses, or healthy and PPID geriatric horses. The PT in PPID horses was longer than healthy geriatrics (18 ± 1.6 seconds versus 16.7 ±0.7 seconds; $p = 0.03$) but not clinically significant. By study design, ACTH and α-MSH were different between groups ($p < 0.001$). PPID geriatrics were older ($p = 0.02$) than healthy geriatric horses. Older horses and horses with PPID do not appear to develop systemic evidence of hypercoagulability as evaluated by laboratory testing.

**ABSTRACT E-21**

**RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS) RESPONSE AND ACTH/ALDOSTERONE RATIO IN SEPTIC NEWBORN FOALS.** KA Dembek[1], K Onasch[1], SD Hurcombe[1], CW Kohn[1], NM Slovis[2], SM Reed[3], RE Toribio[1]. [1]The Ohio State University, College of Veterinary Medicine; Columbus, OH, USA, [2]Hagyard Equine Medical Institute, Lexington, KY, USA, [3]Rood and Riddle Equine Hospital, Lexington, KY, USA

Sepsis is a major cause of mortality in newborn foals. Dysfunction of the hypothalamic-pituitary-adrenal axis (HPAA), manifested as relative adrenal insufficiency (RAI), has been associated with sepsis in foals. The HPAA and RAAS are interactive systems, and a relationship between RAAS activation and RAI is well documented in critically ill children, but limited information exists in septic foals. We hypothesized that in septic foals the RAAS will be activated by inflammation and hypoperfusion and the degree of activation will be associated with severity of sepsis and mortality. We also proposed that ACTH will be associated with aldosterone concentrations.

Blood samples were collected on admission from 49 septic (sepsis score > 12), 59 sick non-septic (SNS), and 20 healthy foals of < 3 days of median age. Blood concentrations of cortisol, aldosterone, angiotensin-II (ANG-II), ACTH and plasma renin activity were determined by immunoassays.

Aldosterone, ACTH and cortisol concentrations were higher in septic and SNS compared to healthy foals. Septic foals had higher ACTH/aldosterone and ACTH/cortisol ratios than healthy foals. Nonsurviving septic foals had a higher ACTH/cortisol ratio than survivors. No differences in renin activity and ANG-II concentrations were found, but nonsurviving septic foals had lower renin activity. Hyperkalemia and hypochloremia were correlated with hyperaldosteronemia. Hyperlactatemia was associated with aldosterone, cortisol and ACTH concentrations in septic foals.

RAAS activation in critically ill foals is characterized by increased aldosterone concentrations. The high ACTH/cortisol and ACTH/aldosterone ratios in septic foals suggest that RAI is not restricted to the zona fasciculata but also to the zona glomerulosa.

**ABSTRACT E-22**

**EFFECTS OF BLOOD CONTAMINATION ON PARAMETERS OF CEREBROSPINAL FLUID ANALYSIS.** M Furr. Equine Medical Center, Virginia-Maryland Regional College of Veterinary Medicine, Leesburg, VA

Evaluation of cerebrospinal fluid is a key component of the examination of horses with neurologic disease. Contamination of the sample by peripheral blood during collection is a recognized complication of the procedure, and can compromise the assay of the CSF. To date, it is unclear how to evaluate CSF with blood contamination as the magnitude of the effects have not been quantified. The purpose of the study was to determine the effects of blood contamination on selected CSF parameters, and secondly to test a common correction equation for peripheral blood contamination (i.e. proportionality of RBC to WBC counts in whole blood).

To perform the experiment, peripheral blood from a normal horse was diluted 1:2,000-1:10,000 with PBS and used to spike normal CSF ($N = 6$) to result in mean RBC counts of 34 to 1,450 RBC/ l. Following this, CSF was evaluated using conventional clinical laboratory methods. To test the correction calculation, pooled normal CSF with a WBC count of "0" was used. Aliquots of CSF (500 l) were spiked with WBCs resulting in a concen-

tration of either 100 or 500 WBC/ l. CSF was then again spiked with whole blood from healthy horses (N = 6); samples were prepared in duplicate and analyzed using routine clinical methods. Procedures were approved by the Virginia Tech Institutional Animal Care Committee.

Results demonstrated that between a range of 0 and 1,450 RBC/ml CSF there was no obvious effect upon WBC counts or CSF total protein- these values remained unchanged from normal, uncontaminated CSF. Mean total protein was 40.8 mg/dl in uncontaminated CSF and 52.9 mg/dl in the sample with the highest blood contamination (1,450 RBC/ l). WBC counts were 0 cells/ l in uncontaminated and 0.6 cells/ l in the sample with the greatest blood contamination (RBC count 1,450 cells/ l). CSF samples prepared with high WBC counts, then spiked with peripheral blood demonstrated good correlation between actual cell counts observed and that predicted by the correction equation. However, observed values differed between –10 and + 30% from that expected and a Bland-Altman plot demonstrated limits of agreement up to 150 cells/ l, which was considered to be poor performance.

The conclusions were that RBC contamination of CSF up to a value of 1,500 RBC/ l does not alter resultant WBC or total protein values. RBC counts greater than 10,000 RBC/ l, however are highly correlated with increasing TP and WBC numbers. Further, the standard correction equation for RBC contamination of CSF does not appear to be accurate enough to recommend its routine clinical use.

**ABSTRACT E-23**

**THE EPIDEMIOLOGY OF SHIVERS IN HORSES.** A Draper[1], J Bender[1], AM Firshman[1], JD Baird[2], S Reed[3], IJ Mayhew[4], SJ Valberg[1]. [1]College of Veterinary Medicine, University of Minnesota, St Paul MN, [2]Ontario Veterinary College, Guelph, ON, Canada, [3]Rood and Riddle Equine Hospital, Lexington KY. 4. Massey University, Massey, New Zealand

Shivers is a poorly defined, chronic equine movement disorder. This study sought to characterize the epidemiology of Shivers.

A standardized web-based survey was used to collect data on signalment, onset, clinical signs, precipitating factors, effect of management, diet and therapies. A standardized video was requested from owners, where possible. Shivers was defined as hyper-flexion or -extension of the pelvic limbs during backwards walking and manual hoof lifting. Diagnosis was confirmed either by video submission [V+], or by reported clinical signs without video [V-]. Two matched case-controls were obtained from V+ owners that were normal, > 4 years old and in close proximity. Analysis was by $Chi^2$ analyses, $p < 0.05$.

Responses included; V+ n = 32, V- n = 62, controls n = 47. V + had significantly more WBs, TBs and Draft breeds, taller horses (>17hh) and males compared to the controls. Signs frequently began at <10 years of age (V+ and V-). Clinical signs in V+/V- horses included farrier problems (88%/91%), muscle twitching (88%/84%), muscle wasting (44%/33%), weakness (31%/22%) and exercise intolerance (25%/14%). Both groups reported an improvement with increased turnout, exercise and a low carbohydrate diet whereas NSAIDs, muscle relaxants, chiropractic and acupuncture therapy had minimal effect. 50% of V+ horses showed worsening of clinical signs over time, and with stress compared to V- horses.

In conclusion, Shivers often begins before 10 years of age, is more common in male horses over 17hh, shows progressive signs in 50% of cases and is best managed by increased turnout, regular exercise and a low carbohydrate diet.

**ABSTRACT E-24**

**PROGNOSTIC FACTORS ASSOCIATED WITH SHORT-TERM AND LONG-TERM OUTCOME IN HORSES WITH CHRONIC RENAL FAILURE.** N Nogradi, B Toth, MB Whitcomb, R Riley, N Pusterla. William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, CA

Chronic renal failure (CRF) is considered a devastating clinical syndrome in horses, however prognostic factors have not been determined. The objective of the present study was to describe the most common clinical, clinicopathologic and utrasonographic findings in horses diagnosed with chronic renal failure and to reveal prognostic factors associated with short-term (discharge from hospital) and long-term (12 months) survival.

Medical records of 58 horses diagnosed with CRF were reviewed. Information on signalment, history, clinical examination findings, laboratory testing, renal ultrasonography and outcome were collected. Long-term outcome was determined by telephone interviews with owners. Statistical analysis included the formulation of multivariate logistic regression models.

Thirty-eight horses (65.5%) were discharged from the hospital after diagnosis and initial treatment. Long-term follow up was available for 24 cases and 14 horses were alive at 12 months after discharge. Both short-term and long-term nonsurvivors had a significantly higher serum creatinine and blood urea nitrogen concentration when compared to survivors ($P < 0.01$ in both cases) and they more commonly had decreased kidney size detected by ultrasound examination ($P = 0.01$ and $P = 0.02$ respectively). Based on the results of the multivariate logistic regression model, the serum creatinine concentration, the presence of hyponatremia and a decreased kidney size on admission were associated with a negative short-term outcome, while no factors were found to be associated with long-term outcome.

Both laboratory and ultrasound findings are important to assess short-term prognosis in horses with CRF, but admission findings do not determine long-term outcome once the horse is discharged form the hospital.

**ABSTRACT E-25**

***IN VIVO* ADMINISTRATION OF CPG ENHANCES FOAL NEUTROPHIL FUNCTION.** ND Cohen[1], JR Nerren[1], AI Bordin[1], CC Love[1], CE Brake[1], M Liu[1], KR Kuskie[1], MH Kogut[2]. [1]College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, [2]USDA-ARS-sparcffsru, College Station, TX

Neonates are particularly dependent on innate immune responses for protection against infection during early life because of deficiencies in adaptive immunity. Neutrophils are primary effector cells of the innate immune response; however, deficiencies in functional responses of neonatal neutrophils exist. Consequently, enhancing neutrophil function in neonates might help protect newborns against infectious diseases. The goal of this project was to determine whether *ex vivo* neutrophil function of neonatal foals could be stimulated by *in vivo* administration of unmethylated CpG oligodeoxynucleotides, agonists for Toll-like receptor 9 (TLR9). CpGs were administered IM to 9 foals on days 1 and 7 of life; an equal volume of saline was administered IM to 9 control foals on days 1 and 7. Neutrophils were isolated from blood collected from foals on days 1, 3, 7, 9, 14, and 28. Neutrophil degranulation was significantly ($P < 0.05$) lower on days 3, 9, and 14 for CpG-treated foals than for control foals. Neutrophils from CpG-treated foals stimulated with *Rhodococcus equi* expressed significantly greater interferon-gamma (IFN-γ) mRNA on days 3, 9, and 14 than neutrophils from control foals; at age 28 days, there were no significant differences between treatment groups in expression of IFN-γ. Reactive oxygen species generation was not affected by CpG administration. Collectively these results suggest that administration of CpG to neonatal foals may enhance neutrophil function during the first fortnight of life. The increased expression of IFN-γ in response to stimulation may be of particular importance because neonatal foals have been shown to be IFN-γ-deficient at birth.

**ABSTRACT E-26**

**EFFECT OF AGE ON THE PHARMACOKINETICS OF A SINGLE DAILY DOSE OF GENTAMICIN SULPHATE IN FOALS.** AJ Burton[1], S Giguère[1], Y Al Hamhoom[1], L Warner[1], RD Arnold[1]. [1]University of Georgia, Athens, GA

Therapeutic drug monitoring in a small number of foals indicates that the standard adult dose of 6.6 mg/kg q 24 h for gentamicin is too low and a dose of 12 mg/kg has been proposed. The

pharmacokinetics of this dosage in foals and the ages at which this higher dose should be applied have not been investigated. The objective of this study was to determine the effect of age on the pharmacokinetics of a single 12 mg/kg dose of gentamicin in foals.

Six healthy foals were given a single IV dose of gentamicin at ages 1-3 days, 2, 4, 8 and 12 weeks of age. Plasma concentrations of gentamicin were measured using HPLC-MS. Pharmacokinetic data were analyzed using a noncompartmental approach. Effect of age on each pharmacokinetic parameter was assessed using a one-way ANOVA for repeated measures.

Gentamicin concentrations (mean +/- SD) 1 hour after administration were significantly higher at 8 (68.78 +/- 13.45 μg/mL) and 12 (65.05 +/- 22.81μg/mL) weeks of age compared to 1-3 days (20.52 +/- 2.07 μg/mL). Trough concentrations were significantly lower at 4, 8 and 12 weeks of age (all < 0.78 μg/mL) than at 1-3 days (1.97 +/- 0.90 μg/mL), consistent with a significantly longer elimination half-life in 1-3 day-old foals compared to other ages. Effect of age on AUC and clearance was not statistically significant.

These data indicate that age has a profound effect on the pharmacokinetics of gentamicin in foals and support the need for a 12 mg/kg dose in neonatal foals.

## ABSTRACT E-27

**DEMONSTRATION OF ENDOGENOUS INFLAMMATORY CYTOKINE PRODUCTION BY EQUINE ADRENOCORTICAL TISSUE IN AN *EX VIVO* MODEL.** KA Hart, MH Barton, NA Norton, ML Vandenplas. Department of Large Animal Medicine, University of Georgia College of Veterinary Medicine, Athens, GA

Transient adrenal dysfunction characterized by inadequate cortisol responses occurs in 40-60% of septic people and foals. The pathogenesis of this critical illness-related corticosteroid insufficiency (CIRCI) is unknown, but adrenocortical suppression mediated by inflammatory cytokines is believed to be key. Human and bovine adrenocortical cells directly release inflammatory cytokines in response to bacterial endotoxin; this local cytokine production might potentiate adrenal suppression and CIRCI in patients with gram-negative sepsis. The objective of this study was to determine if equine adrenocortical cells directly release inflammatory cytokines in response to bacterial endotoxin. Adrenal tissue was collected from 6 healthy horses after euthanasia and adrenocortical explants were prepared, cultured for 24 hours, and exposed to *E. coli* endotoxin (0.1-10 ng/ml). Supernatants were collected after 1, 2, 4, 8, and 20 hours and assayed for IL-6, IL-10, and TNF-α by ELISA. Cytokine production was expressed as ng/ml/g explant weight. Data was analyzed with analysis-of-variance, with significance at $P < 0.05$. IL-6, IL-10, and TNF-α production was observed in control and treated explants, but IL-10 and TNF-α concentrations (peak mean concentrations 834.1±809.5 ng/ml/g and 694.0±489.1 ng/ml/g respectively) were higher than IL-6 concentrations (peak mean concentration 62.82±147.4 ng/ml/g). Cytokine production was not significantly different between treated and control explants, though a trend toward increased TNF-α production in treated explants was observed. These data illustrate endogenous cytokine production from equine adrenocortical tissue, and suggest that the equine adrenal may act as a local and systemic inflammatory organ. Further study is needed to elucidate links between inflammation and equine adrenal function.

## ABSTRACT E-28

**PROPIONATE AND OTHER METABOLITES IN HORSES PUT ON PASTURE.** ML Katz, DT Lewis. Veterinary & Animal Sciences, University Massachusetts, Amherst, MA

Exposure to readily available grass carbohydrates (NFC) is thought to be a stimulus for laminitis. This experiment exposes horses to higher grass NFC after ingestion of long-term low NFC hay, then to lower grass NFC after long-term ingestion of high NFC hay.

Three miniature horses were fed nothing but ad-libitum first-cut hay plus a vitamin supplement at all times from 2009 through 2011 other than 10 days when on pasture. The same hay lot was fed for at least 4 months prior to each experimental period. The horses were sampled hourly over 2 days in August and 2 days in October 2010 via jugular catheters, eating hay the first day and mixed grass pasture the next. The same horses were sampled twice for 3 days each in both August and October 2011. The 2010 pasture contained at least twice the NFC as in the hay; the 2011 pasture contained ½ to 2/3 the NFC as in the hay. In both years, October pasture contained significantly more water than did August pasture. [NFC as % dry matter in hay: 2010 = 8; 2011 = 23.5 // in pasture: 8/10 = 18.9; 10/10 = 18.9; 8/11 = 11.6; 10/11 = 16.4. H2O % in pasture: 8/10 = 60; 10/10 = 78; 8/11 = 54, 10/11 = 77.]

Glucose concentrations (GLUC) on hay averaged about 90 mg/dl in 2010 and 80 mg/dl in 2011. In 2010, GLUC rose to 120 mg/dl after 5-6h on pasture before returning to baseline. In 2011, GLUC rose by a statistically significant amount only in the October samplings (5-15 mg/dl within 2.5h, soon returning to baseline). Insulin concentrations were constantly low on hay (< 7 uIU/ml, mean = 4). Insulin mean concentrations in 2010 gradually rose on pasture to a peak of about 70 uIU/ml (after 6h in August, 9h in October), then gradually decreased. Insulin rose significantly within 2h in both August 2011 sessions, (peak 15 uIU/ml after 4h), then subsided. A similar rapid increase occurred in October 2011 (peak 160 uIU/ml 1st run; 70, 2d run), then subsided. Mean 2010 propionate concentration (MPC) on hay was .08 mM in August and .06mM in October. This rose immediately in both months and remained elevated as long as the horses were on pasture (12h). The 2011 MPC on hay was .05-.06 mM, rising after 5h on pasture. Peak MPC was reached after 24–26h on pasture. [1st August run = .105 mM; 2d = .085mM; 1st October run = .09mM; 2d = .115mM.] MPC decreased after 28 hours in all but the 1st August run. Lactate and pyruvate concentrations revealed no statistically significant changes. However, the mean lactate/pyruvate ratio (L/P) significantly increased on pasture in both years. Basal mean L/P was about 20 in 2010, increasing to 40 within 1h on pasture, and peaking at 70 by the end of the sampling period (12 h). The 2011 basal level was about 25, rising to 35 by the 1st sampling in all pasture sessions. L/P/peak values (35–50) were reached by 2.5h in the 1st Aug session and by 5h in the other sessions. L/P remained only moderately elevated thereafter and returned to baseline by Day 3 in all 2011 sessions.

## ABSTRACT E-29

**EFFECT OF DIETARY CARBOHYDRATE CHALLENGE ON ACTIVATION OF 5'-ADENOSINE MONOPHOSPHATE ACTIVATED PROTEIN KINASE (AMPK) IN LIVER, SKELETAL MUSCLE, AND DIGITAL LAMINAE OF LEAN AND OBESE PONIES.** TA Burns[1], MR Watts[1], RJ Geor[2], LJ McCutcheon[2], JK Belknap[1]. [1]College of Veterinary Medicine, The Ohio State University, Columbus, OH, [2]College of Veterinary Medicine, Michigan State University, East Lansing, MI

Systemic insulin resistance and hyperinsulinemia are proposed to be important risk factors for laminitis associated with equine metabolic syndrome (EMS) in horses and ponies. AMPK, a highly conserved enzymatic regulator of cellular energy status, has become a therapeutic target for human MS and EMS due to its reported ability to increase systemic insulin sensitivity. For example, the AMPK agonist metformin has recently been used clinically to treat EMS-affected equids. However, regulation of AMPK activity in both 1) tissues primarily responsible for insulin-mediated glucose disposal (liver, skeletal muscle) and 2) the target tissue for injury in EMS-related laminitis, the digital laminae, is largely unknown. The purpose of this study was to characterize the cellular localization and activation state of AMPK in liver, skeletal muscle, and digital laminae of ponies subjected to a dietary carbohydrate challenge meant to mimic abrupt exposure to pasture

rich in nonstructural carbohydrate (NSC). Following 4 weeks of conditioning consisting of a diet of hay chop (NSC ˜6% on a DM basis), mixed-breed ponies (body weight 270.9 +/- 74.4 kg) were assigned to groups based on body condition scoring (lean vs. obese). Ponies either remained on the conditioning diet (CON diet; n = 5 obese, n = 5 lean) or received the same diet supplemented with sweet feed and oligofructose (CHO diet [˜42% NSC]; n = 6 obese, n = 6 lean) for a period of 7 days. At the end of the feeding protocol, samples of dorsal digital laminae, middle gluteal muscle, and liver were collected immediately following euthanasia; samples were formalin-fixed or snap frozen. AMPK was primarily localized to keratinocytes and vascular elements in the laminae and hepatocytes (vs. local vascular elements) in the liver via total AMPK immunohistochemistry. Western blot analysis for phospho(P)-AMPK (indicating activated AMPK) demonstrated decreased laminar P-AMPK concentrations upon challenge with a high-carbohydrate diet ($p = 0.01$). In contrast, P-AMPK concentrations were unchanged in skeletal muscle ($p = 0.33$), and there was a trend for increased AMPK activation in the liver in obese ponies in response to dietary CHO challenge ($p = 0.13$). Unchanged or increased P-AMPK concentrations in the setting of increased caloric intake suggest insulin resistance in skeletal muscle and liver; the decreased laminar P-AMPK concentrations with CHO challenge indicate that laminar tissue remains insulin sensitive. In conclusion, whereas skeletal muscle and liver are likely contributory to systemic insulin resistance and resulting hyperinsulinemia in EMS, laminar dysfunction/injury in EMS is more likely due to the local effects of hyperinsulinemia, and not due to local insulin resistance/energy failure.

## ABSTRACT E-30

**REPEATABILITY OF THE COMBINED GLUCOSE AND INSULIN TEST IN HORSES.** J Bröjer, K Alvarsson, J Hedenskog, K Nostell. Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, Sweden

The combined glucose-insulin test (CGIT) has been applied as a dynamic test for insulin sensitivity in horses. However, there is limited information on the reproducibility of the test. The object of the study was thus to evaluate the repeatability of calculated variables derived from the CGIT glucose and insulin curves in horses.

Eighteen healthy horses (9 Icelandic horses and 9 Standardbred horses; age 6 – 20 years) underwent the CGIT on two occasions with 3 weeks between the tests. The area under the glucose and insulin vs. time curves (AUC-glu and AUC-ins), the duration of the positive phase of the glucose curve (DPP-glu), and the time duration to reach the insulin concentration 100 mU/L (DIC-100) were determined. There were no significant differences between the two tests ($p < 0.05$). The interday variation expressed as the coefficient of variation (CV) and intraclass correlation coefficient (ICC) were calculated. The CV and ICC was 14.6 % and 0.64 for the AUC-glu and 7.7 % and 0.88 for the AUC-ins. The DPP-glu had CV and ICC values of 44.9 % and 0.61, whereas the corresponding values for DIC-100 were 7.0 % and 0.88.

When the CGIT is used as a diagnostic test to evaluate insulin resistance, the patient is considered to be insulin resistant when the DPP-glu > 45 min or the insulin concentration > 100 mU/L at 45 min. However, the large CV and low ICC for DPP-glu in the present study suggests that normal or impaired insulin sensitivity should not be defined solely on this parameter.

## ABSTRACT E-31

**DOPAMINE AND TYROSINE HYDROXYLASE CONCENTRATIONS IN PARS INTERMEDIA TISSUE OF HORSES WITH PITUITARY PARS INTERMEDIA DYSFUNCTION WITH AND WITHOUT PERGOLIDE TREATMENT.** HC Schott II[1], JS Patterson[2], EB Howey[2], MJ Benskey[3], JL Goudreau[3], KJ Lookingland[3]. [1]Department of Large Animal Clinical Sciences; and [2]Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine; and [3]Department of Pharmacology and Toxicology, Neuroscience Program; Michigan State University, East Lansing, MI

Pituitary pars intermedia dysfunction (PPID) develops slowly over a period of years as degeneration of hypothalamic dopaminergic neurons leads to proliferation of pars intermedia (PI) melanotropes through hyperplasia and adenoma formation. Further, concentrations of dopamine (DA) and DA metabolites, as well as tyrosine hydroxylase (TH) immunoreactivity, are markedly reduced in PI tissue of PPID-affected equids. Treatment with the DA receptor agonist pergolide results in notable clinical improvement in PPID-affected horses. Thus, we hypothesized that treatment of PPID-affected horses with pergolide would result in greater DA and TH concentrations in PI tissue collected from PPID-affected horses treated with pergolide, as compared to PI tissues collected from untreated PPID-affected horses. To test this hypothesis, pituitary glands were removed within 30 min of euthanasia (Aug-Nov, 2009) from 18 horses: four untreated PPID-affected horses (28±4 [SD] years), four aged (25±5 years) and four young (5±2 years) control horses without signs of PPID, and six PPID-affected horses (25±3 years) that had been treated with pergolide for 6 months. PPID status was confirmed by dexamethasone suppression test results. Pituitary glands were sectioned in a midline sagittal plane with one half rapidly frozen on dry ice and the other half placed in formalin. Using histological findings to outline the PI, an 18 g needle was used to collect core samples of PI tissue from the opposing half of pituitary glands, after thawing. After tissue sonification, supernatant fractions were analyzed for DA concentration by high performance liquid chromatography and standardized to protein concentration in the remaining cell pellet (reported as ng DA/mg protein). TH protein concentration (normalized to β-actin) was determined by Western blot analysis. DA concentration was greatest ($p < 0.05$) in PI tissue collected from young control horses, as compared to tissues collected from the three other groups of horses. In contrast, TH concentration in PI tissue was not different between PPID-affected horses treated with pergolide and both young and aged control horse PI. However, PI TH concentration was lowest ($p < 0.05$) in untreated PPID-affected horses.

In conclusion, pergolide treatment of PPID-affected horses appears to restore TH concentration in PI melanotropes. Further study is needed to determine whether the persistently low DA concentrations in PI tissue of pergolide treated horses is a real finding or a potential artifact of tissue collection and processing.

## ABSTRACT E-32

**EFFICACY OF OMEPRAZOLE PASTE IN THE PREVENTION OF GASTRIC ULCERS IN HIGH LEVEL ENDURANCE HORSES.** Y Tamzali[a], LM Desmaizieres[b], C Marguet[b], M Birague[b], F Lyazrhi[c]. [a]Equine Internal Medicine, INP-Ecole Nationale Vétérinaire, Toulouse, France, [b]La Clinique du Cheval, Grenade, France, [c]Educational unit of biostatistics, National Veterinary School, Toulouse, France

A high EGUS prevalence was shown in elite endurance horses in a recent study with 48% during interseason period compared to 93% during the competition season with a significant influence of training and performance level on the gastric score. According to these results it was hypothesized that omeprazole preventative treatment may reduce the gastric scores in endurance horses maintained in similar training and performance conditions. 26 endurance horses competing at high level were selected and submitted to two gastroscopic examinations. The first gastroscopy was performed during training period 4 weeks before a planned ride of 90 km minimum and up to 160 km. Subsequently horses received 1mg/kg bwt omeprazole paste (Gastrogard) once daily, in the morning for 28 days. The second gastroscopy was performed within two-three days following the ride. Data related to housing, feeding, training system as well as age, breed and gender were recorded for each horse. The prevalence of squamous gastric ulcers was 76.9% during the training period (mean score 1.038 ± 0.87 on a scale from 0 to 4) and 73% following the ride (mean score 0.884 ± 0.76)

with no significant difference ($p = 0.749$ for prevalence and $p = 0.103$ for gastric scores). There was no influence of ride distance on the gastric score (in a previous prevalence study in non-treated horses it was shown that the distance of the ride preceding the second gastroscopy had a significant influence on the gastric score). There was a significant influence of housing on the gastric score ($p = 0.018$) showing lower scores in the horses housed in a mixed environment (stable + pasture) versus horses kept totally on pasture. There was also a significant influence of age on the gastric scores ($p = 0.042$). Breed or gender had no influence on gastric scores. This study suggests that omeprazole paste, 1 mg/kg bwt *peros* is effective in preventing worsening of gastric scores EGUS in endurance horses.

## ABSTRACT E-33

**A COMPARISION OF TWO DOSES OF OMEPRAZOLE IN THE TREATMENT OF GASTRIC ULCERATION IN THOROUGHBRED RACEHORSES.** BW Sykes[1], GD Hallowell[2]. [1]Upper Orara, NSW, [2]University of Nottingham, Sutton Bonington, UK.

Equine gastric ulcer syndrome (EGUS) is a well recognized condition in Thoroughbred racehorses. The purposes of this study were to compare the therapeutic efficacy of two doses of omeprazole, and to report the healing rate of glandular EGUS with omeprazole therapy. Thoroughbred racehorses in training with grade II-IV/IV glandular EGUS were identified on gastroscopic examination. Squamous EGUS score was also recorded. Horses were randomised to receive either 2.0 gram (high dose) or 0.8 gram (low dose) omeprazole PO SID, equivalent to 4.0 and 1.6 mg/kg PO SID respectively for a 500 kg horse. Repeat gastroscopy was performed at 33± 7 days and squamous and glandular scoring repeated. Twenty-four horses met the inclusion criteria and were randomised into two equal groups. One horse in the low dose group was lost to follow up for unrelated reasons. All horses, except one in the high dose and one in the low dose group, had grade II-IV/IV squamous ulceration at day 0. Data were analysed using McNemar's Test, Mann Whitney U and Wilcoxon Paired Tests. Significance was assumed at $p < 0.05$. No differences between the groups at enrolment, or in the duration of therapy, were present ($p > 0.38$). Ulcer healing (defined as a return to grade 0-I/IV) occurred in 67% and 44% of the squamous, and 50% and 11% of the glandular, ulcers in the high and low dose groups respectively. A dose effect for healing was noted for both squamous and glandular ulcer healing ($p = 0.003$ and $p < 0.0001$ respectively) whereas a dose effect for improvement was only seen for squamous ulcers ($p = 0.03$; glandular $p = 0.13$). A relationship between healing of squamous and glandular ulcers, wherein glandular healing, or improvement, was more likely to have occurred if squamous healing, or improvement, had occurred, existed in the high dose, but not low dose group ($p = 0.005$ and $p = 0.10$ for healing, and $p = 0.04$ and $p = 0.12$ for improvement, for high and low dose respectively).

In conclusion, healing of EGUS is commonly incomplete at 33 days and a dose dependent response exists. Control gastroscopy is indicated to ensure healing has occurred, even when a 28 day course has been completed, and a reduction in the omeprazole dose below 4 mg/kg PO SID prior to this is likely to result in inadequate healing in a significant number of patients. Healing of glandular ulceration is inferior to healing of squamous ulceration and the current recommendation of 28 days therapy is inadequate. Investigation of complimentary and/or alternative therapies for glandular EGUS is warranted as the response to omeprazole therapy alone is poor.

## ABSTRACT E-34

**EFFECT OF EGUSIN® 250 AND SLH ON GASTRIC ULCER SCORES, GASTRIC FLUID PH AND TOTAL CARBON DIOXIDE IN HORSES.** MC Woodward[1], NK Huff[1], F Garza Jr[1], ML Keowen[1], MT Kearney[2], FM Andrews[*,1]. [1]Equine Health Studies Program, Department of Veterinary Clinical Sciences and, [2]Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA

Gastric ulcers are common in horses, but current pharmaceutical treatments are expensive and require prescriptions. EGUSIN® 250 (E-250) and EGUSIN® SLH (E-SLH) are commercially available feed supplement for the treatment and prevention of gastric ulcers, but data on their effectiveness has not been reported. The purpose of this study was to evaluate these two formulations for the treatment and prevention of gastric ulcers in stall-confined horses undergoing feed-deprivation.

This study was a blinded two-period partial cross-over study using Nine Thoroughbred and Thoroughbred-cross horses (five geldings and four mares). Treatments consisted of an untreated control and two treatment groups (E-250 and E-SLH) mixed with a sweet feed. Horses were confined to a stall and treated for three weeks, followed by a one-week alternating feed-deprivation period, and then for one week after the feed-deprivation period (five total weeks of treatment). Gastroscopies were performed on horses on day 0 and at the end of week 3, week 4 and week 5. Gastric juice was aspirated and pH was measured. Gastric ulcer number and severity scores were assigned at each gastroscopy. Venous blood gas samples were obtained to determine changes in total $CO_2$ and due to bicarbonate in the supplements. A repeated measures ANOVA using the mixed procedure in SAS was used to analyze the data. When significant differences were found, post-hoc pairwise comparisons were conducted with t tests of least-squares means for main effects and for interaction effects. A $P < 0.05$ was considered significant.

Non-glandular gastric ulcer scores significantly increased in all horses from Week 3 to Week 4, as a result of feed-deprivation. There was no significant treatment effect on non-glandular gastric number or severity scores through four weeks of study. However, E-SLH and E-250 treated horses had significantly lower nonglandular ulcer number and severity scores at week 5. There was no significant difference in gastric juice pH between treatment groups at any time during the study. $TCO_2$ was significantly increased in the E-250 and E-SLH treated horses at 24 hours when compared to the 12 hour samples.

No significant treatment effects were seen in the first four weeks of the study. However, one week after feed-deprivation, EGUSIN®-treated (E-SLH and E-250) horses had fewer and less severe gastric ulcers. Supplementation with EGUSIN® products may have efficacy in improving gastric ulcers in stall-confined horses undergoing intermittent feeding.

## ABSTRACT E-35

**EVALUATING REPLACEMENT OF SUPPLEMENTAL INORGANIC MINERALS WITH ZINPRO PERFORMANCE MINERALS ON PREVENTION OF GASTRIC ULCERS IN HORSES.** P Loftin[1], M Woodward[1], W Bidot, J Cartmill[1], S Zoccarato, F GarzaJr[1], M Keowen[1], C Larson[2], FM Andrews[1]. [1]Louisiana State University, Baton Rouge, LA, [2]Zinpro Corporation, Eden Prairie, MN

Non-glandular (NG) gastric ulcers are common in the horse. Zinc is an essential co-enzyme involved in healing of mucosal surfaces and has been used to treat and prevent gastric ulcers in other species. The purpose of this study was to test the efficacy of a feed supplement containing zinc-methionine and other organic minerals (ZPM) to a supplement containing inorganic zinc, as zinc sulfate (ZS), for prevention of gastric ulcers in horses.

The study was a blinded 2-period non-crossover using adult Thoroughbred horses ($n = 36$). ZPM (400 mg) or ZS (400 mg) milled into pellets was mixed with crimped oats and fed to the horses twice daily for 56 days. Horses were stratified by NG ulcer score, then by sex and assigned to two treatment groups ($n = 18$). There were two 56-day periods, where horses were stall-confined in ambient environmental conditions. Horses received omeprazole paste (GastroGard®, 4 mg/kg, PO, Q24h) from days 1-14, were subjected to alternating feed-deprivation from days 42-49 and allowed to recover for 7 days. Gastroscopy was performed on

days 0, 14, 42, 49, and 56. Gastric juice pH was measured and gastric ulcer number and severity scores were assigned by a mask clinician (FMA). Weather data were collected for the two periods. Gastric ulcer scores were expressed as mean (SD) and an ANOVA for repeated measures was used to compare ulcer scores between groups and over time. A post-hoc Tukey's test was used to determine differences ($P < 0.05$).

Horses ($n = 34$) were treated for all 56 days. There was no treatment effect in the study; however there was a period effect. Mean NG number and severity scores were significantly lower in the ZPM-treated horses, compared to ZS-treated horses, on days 42, 49 and 56, during period 2. Overall, for both periods, mean NG gastric ulcer scores significantly decreased in both treatment groups by day 14, after omeprazole treatment, compared to days -1, 42, 49 and 56. Mean NG gastric ulcer scores significantly increased on day 42, compared to day 14, but were still lower than on day -1. Mean NG gastric ulcer scores increased significantly by day 49, due to alternating feed-deprivation, then decreased to below pretreatment values by day 56. No significant differences were seen in glandular ulcer scores between groups. Means environmental temperature (68ºF vs. 84ºF), heat index (82 vs. 90) and relative humidity (74% vs. 79%) were higher during period 2 of the study and may have contributed to the effectiveness of the ZPM.

ZPM-treatment resulted in lower gastric ulcer scores in horses participating in period 2 of this study, but when data were pooled there was no treatment affect. ZPM at levels fed in this study may be beneficial in preventing gastric ulcers after omeprazole treatment in horses housed in stalls and fed intermittently, especially in hot and humid environmental conditions.

## ABSTRACT E-36

**EVALUATION OF THREE GASTROINTESTINAL PROTECTANTS IN A RODENT MODEL OF EQUINE CANTHARIDIN TOXICOSIS.** HJ Qualls[1], TC Holbrook[2], LL Gilliam[2], B Njaa[2], R Panciera[2], C Pope[2], M Payton[2]. [1]Washington State University, Pullman, WA, [2]Oklahoma State University, Stillwater, OK

The objective of this study was to evaluate the efficacy of three gastrointestinal protectants utilizing rats in an experimental model of equine cantharidin toxicosis.

Male Sprague-Dawley rats were surgically implanted with telemetry devises to record heart rate, locomotor activity and body temperature. Combinations of cantharidin and treatments (mineral oil, activated charcoal, and smectite) were administered via orogastric gavage. Data were recorded for 24 hours prior to and after treatments, and urine was collected for cantharidin analysis. Rats were sacrificed 24 hours after treatment and necropsy was performed. Data analysis included a one-way ANOVA and Fischer Exact Tests.

Cantharidin intoxicated (CI) rats treated with mineral oil had a significantly lower survival rate than all treatment groups. (cantharidin ($p = 0.018$), charcoal ($p = .001$), smectite ($p = .001$)). There was a significant decrease in mean heart rate (328bpm; control - 407bpm) and temperature (30.6°C; control - 36.5°C) in mineral oil treated CI rats during the first 8 hours of treatment ($P < 0.05$). The mean temperatures of charcoal treated rats were significantly lower than the control groups only at the 8-12 hour post-intoxication interval. Mineral oil treated CI rats had the highest mean urine cantharidin concentration. ($P = < 0.05$).

These results suggest mineral oil increases cantharidin absorption resulting in higher morbidity and mortality in rats. Both charcoal and smectite appear to reduce morbidity in this rat model of cantharidin toxicosis.

## ABSTRACT E-37

**EFFECT OF N-BUTYLSCOPOLAMMONIUM BROMIDE (BUSCOPANTM) ON EQUINE ILEAL SMOOTH MUSCLE ACTIVITY *EX VIVO*.** KA Hart, CE Sherlock, AJ Davern, T Lewis, TP Robertson. Department of Large Animal Medicine, University of Georgia College of Veterinary Medicine, Athens, GA.

N-butylscopolammonium bromide (NBB) is an anticholinergic agent used to treat spasmodic colic in horses. Intestinal smooth muscle spasm also occurs in horses with intraluminal intestinal obstructions such as ileal impactions, and may decrease lumen diameter in such cases. The antispasmodic effects of NBB may be useful in managing ileal impactions, but the effects of NBB on equine ileal smooth muscle are unknown. The objective of this study was to investigate effects of NBB on equine ileal smooth muscle activity using an *ex vivo* model. Ileal tissue was collected from 6 healthy horses after euthanasia, and isolated circular and longitudinal smooth muscle was mounted in quadruplicate in organ baths connected to isometric force transducers. After equilibration, the effect of NBB (1nM-100μM) on spontaneous and induced contraction was determined and compared to responses in control tissue. At $\geq 30\mu M$, NBB inhibited spontaneous contractions in all tissue segments that exhibited spontaneous activity. NBB inhibited carbachol-induced contraction in circular (NBB-treated EC50 $1.7 \times 10^{-6}$ vs. control EC50 $1.5 \times 10^{-7}$) and longitudinal smooth muscle (NBB-treated EC50 $8.5 \times 10^{-6}$ vs. control EC50 $2.0 \times 10^{-8}$). Abolishment of carbachol-induced contraction with NBB was observed at 4.5-fold lower concentrations in circular than longitudinal smooth muscle. Pretreatment with NBB inhibited carbachol-induced contraction, as NBB-treated tissue required 7.5-fold greater carbachol concentrations to produce sustained contractions than control tissue. Histamine-evoked contraction was not affected by NBB. NBB inhibits spontaneous and cholinergically-mediated contraction in equine ileal smooth muscle *ex vivo*, and may reduce intestinal spasm in equine ileal impactions. Further study is needed to confirm these effects *in vivo*.

## ABSTRACT E-38

**EFFECTS OF SEDATION WITH XYLAZINE OR ACEPROMAZINE ON THROMBOELASTOMETRY AND ON STANDARD EQUINE COAGULATION PARAMETERS.** B Saas[1], K Lascola[1], S Smith[2], G Zyrkowski[1]. [1]College of Veterinary Medicine, Urbana, IL, [2]College of Medicine, Urbana, IL

Acepromazine and xylazine are administered to horses to reduce anxiety, provide analgesia and enable safe evaluation of the animal. These medications may cause a decrease in hematocrit. Variation in hematocrit can interfere with thromboelastometry (TEM). The purpose of this study was to evaluate changes in thromboelastometry (TEM) and standard coagulation assays in horses after the administration of acepromazine and xylazine.

Using a randomized crossover design 7 healthy adult horses were administered a single dose of xylazine (1.1 mg/kg IV) or acepromazine (0.1 mg/kg IV) with a 3 week washout period between treatments. Hematocrit, total protein (TP), platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), TEM and thrombin-antithrombin complexes (TAT) were measured at baseline, 30 minutes and two hours for both medications and at 6 and 12 hours for acepromazine. Repeated Measures ANOVA and Friedman Rank Sum tests were used to evaluate changes in measured parameters with significance set at $p < 0.05$.

At 30 minutes post xylazine administration hematocrit and TP decreased by 20.2% ($p = 0.004$) and 4.3% ($p = 0.013$) respectively. At 2 hours post acepromazine administration hematocrit and TP decreased by 19.7% ($p < 0.001$) and 4.3% ($p = 0.005$) respectively. Changes in TEM included decreased clotting time (Ex-TEM $p = 0.034$) and increased alpha angle (Ex-TEM $p = 0.034$, In-TEM $p = 0.016$) at 30 minutes for horses administered xylazine. No other changes in coagulation parameters were identified.

Changes in TEM parameters after xylazine administration suggest hypercoagulability. The changes in TEM may represent an ex-vivo artifact due to reduced red cell mass rather than a true alteration in coagulation.

## ABSTRACT E-39

**EFFECT OF SEDATION ON ABDOMINAL PRESSURES IN NORMAL STANDING HORSES.** VHL Scott, JL Williams, MC Mudge, SDA Hurcombe. The Ohio State University, Columbus, OH.

Sustained increases in intra-abdominal pressure (IAP) lead to intra-abdominal hypertension (IAH), decreased abdominal perfu-

sion pressure (APP) and abdominal compartment syndrome (ACS); a condition associated with high morbidity and mortality in critically ill people. Horses with colic share similar risk factors to those identified in humans with ACS, which may contribute to unfavorable outcomes seen in some horses with abdominal disease. Sedative administration is employed in people to reduce elevated IAP and may offer a similar therapeutic target in horses with colic. The purpose of this study was to investigate the effect of sedation on direct IAP (dIAP) and APP in standing horses.

Nine healthy adult horses were included in this study. dIAP was measured via abdominal cannulation and electronic sphygmomanometry from the left flank (LFl), right flank (RFl) and ventral (V) abdomen in unsedated horses (baseline), five minutes after low dose xylazine (LD; 0.3 mg/kg IV), and 5 minutes after high dose xylazine (HD, 0.7 mg/kg; total dose 1 mg/kg). Direct mean arterial blood pressure (MAP) was obtained concurrently to calculate APP (MAP-IAP). Mean values of IAP/MAP/APP measured in triplicate were used for statistical analysis. Differences in IAP/MAP/APP for each abdominal site and level of sedation were compared by repeated measures ANOVA. $P < 0.05$ was significant.

LFl dIAP was significantly higher at baseline (-3 mmHg) compared to LD and HD (-5 mmHg for each); $P = 0.023$. LFl APP was not different at any level of sedation (baseline and LD = 105 mmHg, HD = 112 mmHg). RFl dIAP was -5 mmHg at baseline, LD and HD. RFl APP was not different at any level of sedation (baseline = 107 mmHg, LD = 105 mmHg, HD = 108 mmHg). Ventrum dIAP was 25 mmHg at baseline, LD and HD. Ventrum APP was not different at any level of sedation (baseline = 78 mmHg, LD and HD = 77 mmHg). There was no difference in MAP at baseline, LD or HD (102 mmHg, 101 mmHg and 105 mmHg respectively).

Intravenous sedation with xylazine did not affect MAP/APP in normal standing horses, or dIAP when measured from the RFl or V abdomen. Reasons for decreased LFl dIAP observed after sedation are unknown but may be due to differences in abdominal organ topography. Further investigation is required to assess the effect of sedative drugs on dIAP in horses with documented IAH.

## ABSTRACT E-40

**RACING PERFORMANCE AND EARNINGS IN THOROUGHBREDS AFTER COLIC SURGERY: A RETROSPECTIVE COHORT STUDY.** JE Tomlinson[1], RC Boston[1], T Brauer[2]. [1]Department of Clinical Studies - New Bolton, University of Pennsylvania, Kennett Square, PA, [2]Chino Valley Equine Hospital, Chino Hills, CA

Currently there is limited information regarding post-operative performance after abdominal exploratory celiotomy in athletic horses. The null hypothesis of this study was that undergoing colic surgery with or without intestinal resection would not affect subsequent racing performance in Thoroughbred horses. This retrospective cohort study included racing Thoroughbreds that survived to discharge following exploratory surgery for acute colic at Chino Valley Equine Hospital between 1996 and 2010. For each case, 2 matched controls were identified from the last race before surgery. Race earnings, starts, and earnings per start were compared by 6 month periods for up to 42 months following surgery. Associations were explored using mixed effects modeling and regression analysis.

A total of 96 cases were identified; of these 36 (37.5%) had primarily small intestinal lesions, of which 12 underwent resection; 60 (62.5%) had large intestinal lesions, of which 2 underwent resection. In total, 62% of horses that underwent colic surgery returned to racing in the subsequent 42 months. Controls were more likely to race >6 months after surgery than cases (74% versus 62%, respectively, $P = 0.02$). In the 36 months following a 6 month post-surgery lay-up, control horses earned on average $12631 more ($P = 0.18$) and had a mean of 1.7 more starts ($P = 0.15$). This resulted in controls earning a mean of $160 per start more than cases ($P = 0.90$). In conclusion, although horses that underwent colic surgery were less likely to race, those that raced did not show a significant reduction in number of starts or earnings compared to controls.

## ABSTRACT E-41

**PROGNOSIS IN EQUINE COLIC: A RETROSPECTIVE STUDY OF 392 CASES: SHORT-TERM AND LONG-TERM COMPLICATIONS.** CM SchuhDVM, M Nolf DVM, A Benamou-Smith DVM, PhD, Dipl. ACVIM / ECEIM. Equine Center, VETAGROSUP LYON, Marcy L'Etoile, FRANCE.

The purpose of the study is to provide clear data on complications after a surgical or medical treatment of colic.

Three hundred and ninety-two horses admitted to the Equine Clinic ( Clinequine) of the Lyon Vet School (France) for gastrointestinal colic were used in this retrospective study.

History, clinical findings, treatments, outcomes, and complications of 392 colic cases were reviewed. Long-term follow-up data were collected. Complications were described and complication rates were calculated.

The most frequent short-term complication was recurrent colic in both medical and surgical cases ( respectively: 5.7% and 22.4%). Thrombophlebitis (21.7%), incisional infection (18.9%), ileus ( 17.5%), diarrhea (14.7%) and pyrexia ( 14%) are frequently recorded after laparotomy for colic. Diarrhea ( 5.7%) and thrombophlebitis ( 5.1%) are the most common complications, second to recurrent colic, in horses treated medically for colic.

In conclusion, there is still some uncertainties and discrepancies between studies regarding long-term and also short-term complications following colic. Although types of complications are clearly described after a colic treatment, a clear consensus needs to be reached on clinical definitions of each complication, in order to be able to compare results and draw useful conclusions for clinicians.

## ABSTRACT E-42

**PHYSICOCHEMICAL INTERPRETATION OF ACID-BASE ABNORMALITIES IN 54 HORSES WITH ACUTE COLITIS AND DIARRHEA.** DE Gomez[1], LG Arroyo[2], HR Stämpfli[2], LE Cruz[3], OJ Oliver[3]. [1]University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, [2]University of Guelph, Guelph, Ontario, Canada, [3]Universidad Nacional de Colombia, Bogotá, Colombia

Horses with acute colitis often have electrolyte and plasma protein abnormalities which result in significant acid-base imbalances. The quantitative physicochemical approach emphasizes the importance of strong electrolytes (Na, K, Cl, L-lactate), $pCO_2$, and the plasma protein concentrations ($A_{tot}$) in determining plasma pH. The objective of this retrospective study was to demonstrate physicochemical acid-base interpretation of horses with acute diarrhea and to illustrate that different metabolic acid-base situations may occur simultaneously.

Fifty-four horses presented to the Ontario Veterinary College Teaching Hospital during 2008-2010 for clinical colitis and acute diarrhea were included. An admission venous blood sample was collected into a Na-heparin blood gas syringe and analyzed for pH, $pCO_2$ and plasma concentrations of Na, K, Cl, L-lactate and calculated $HCO_3^-$ and Base Excess (BE) using a Radiometer 800 Flex analyzer. Strong ion difference (SID) was calculated as SID = (Na + K)-(Cl + L-lactate), and total protein (TP) was measured using refractometry. Anion gap (AG) and Strong Ion Gap (SIG) were calculated as: AG = (Na + K)-(Cl + $HCO_3^-$); SIG = $A^-$-AG. $A^-$ is net negative charge of plasma proteins; $A^- = 0.22x$ [total protein, g/L]/$(1 + 10^{\{6.65\text{-pH}\}})$. Electro-neutrality (EN) EN = SID-$HCO_3^-$-$A^-$. If BG machine measures accurately and if no additional unmeasured anion is present EN should be zero. Means values were (mean plasma/blood concentrations ± SD): pH = 7.38±0.05; Na = 129±7.3 mEq/L; K = 3.1±0.71 mEq/L; Cl = 94±6.8 mEq/L; $HCO_3^-$ = 24±3.7 mEq/L; BE = -0.22±4.3mEq/L; SID = 35.3±4.4 mEq/L; and Atot = 12.6±3.3 mEq/L; L-lactate = 2.84±2.75 mmol/L; AG = 15.7±5.7 mmol/L; SIG was 2.8±4.8 mmol/L;) EN = -1.40±2 mEq/L. Calculated AG and SIG were significantly correlated with L-lactate concentration AG = 10.4 + 1.36x[L-lactate] ($R^2$ = 0.44), SIG = 1.90-1.17x[L-lactate] ($R^2$ = 0.48). The sensitivity and specificity of AG and SIG to predict hyperlactatemia (L-lactate > 5 mmol/L) was 100% (95% CI 66.4-100) and 84.4% (95% CI 70.5-93.5). The area under the curve (ROC) for the prediction of hyperlactatemia for AG and SIG were 0.956 (95% CI 0.862-0.993) and 0.938 (95% CI 0.838-0.986)

respectively. Physicochemical interpretation of acid base events revealed that metabolic acidosis (strong ion acidosis) and metabolic alkalosis (hypoproteinemic) was concomitantly present in 30% of cases.

These results emphasize the importance of strong ions and proteins in the maintenance of the acid-base balance. AG and SIG predict the presence or absence of clinically significant hyperlactatemia. Acid-base and electrolytes abnormalities were not correlated with the outcome of survival. Electroneutrality was excellent indicating that the measuring system is accurate and that other unmeasured strong anions are unlikely present in significant concentrations in horses with colitis.

**ABSTRACT E-43**

**_IN-VITRO_ GROWTH CHARACTERISTICS OF COMMERCIAL PROBIOTIC STRAINS AND THEIR POTENTIAL FOR INHIBITION OF _CLOSTRIDIUM DIFFICILE_ AND _CLOSTRDIDUM PERFRINGENS_.** A Schoster[1], B Kokotovic[2], A Permin[3], P Dedenroth[4], L Guardabassi[1]. [1]University of Copenhagen, Copenhagen, Denmark, [2]Technical University of Denmark, Copenhagen, Denmark, [3]The DHI group, Copenhagen, Denmark, [4]Clerici-Sacco Group, Cadorago, Italy

Probiotics have gained importance in human and veterinary medicine to prevent enteric disease. Little information is available on commercial probiotic strains regarding their growth characteristics and inhibition of equine enteric pathogens such as *Clostridium difficile* and *Clostridium perfringens*.

To study growth characteristics of 17 commercial probiotic strains (Lactobacilli n = 16, Bifidobacteria n = 1) MRS broth was adjusted to pH 2 or 4 or supplemented with 0.15% or 0.3% bile. Growth was measured at 0 and 24h and compared spectrophotometrically to control growth in standard MRS broth. Growth under aerobic conditions was assessed. To evaluate inhibition of *C. difficile* and *C. perfringens* sterile supernatant of the probiotic culture was added to BHI inoculated with a standard *C. difficile* or *C. perfringens* suspension. Growth was measured spectrophotometrically at 0 and 24h and compared to the control (*C. difficile* or *C. perfringens* suspension in BHI).

At pH 4 12% of strains showed > 50% growth and 24% were unable to grow, however did survive. At pH 2 none of the tested strains grew or survived. Eighty eight percent showed > 75% growth in 0.15% bile, 60% showed > 75% growth in 0.3% bile. Ninety-four percent grew under aerobic conditions. Ninety-four percent of strains were inhibitory (0-20% growth compared to control) against *C. difficile* and 76% were inhibitory against *C. perfringens*.

Sixty percent of the tested strains showed favourable *in-vitro* characteristics for use as potential equine probiotics and could be further studied in placebo controlled clinical trials for prevention or treatment of *C. difficile* and *C. perfringens* associated disease.

**ABSTRACT E-44**

**PLASMA HIGH MOBILITY GROUP BOX-1 LEVELS IN HORSES WITH GASTROINTESTINAL DISEASE.** JR Bauquier, L Nath, G Forbes, SR Bailey. University of Melbourne Faculty of Veterinary Science, Werribee, Victoria, Australia.

High mobility group box-1 (HMGB-1) has been established as a late-phase cytokine in the pathophysiology of sepsis in experimental rodent models and clinical human patients. It typically increases in these species approximately 18 hours after the onset of disease, and shows potential as a therapeutic target. It has been characterized in synovial fluid of horses with joint injury, however its presence has not been investigated in horses with colic and endotoxemia. The aim of this study was to determine whether HMGB-1 is increased in horses with colic, and specifically those with lesions more likely to cause endotoxemia, such as strangulating lesions or colitis. A commercial ELISA kit was used to measure HMGB-1 levels in plasma samples taken at admission from 32 horses presented for colic and 22 normal control horses. HMGB-1 was significantly higher in horses with colic (median 12.2 ng/ml, 95% CI 13.1-21.8 ng/ml) than controls (median 3.5 ng/ml, 95% CI 2.6-5.6 ng/ml), $P < 0.0001$. When colic horses were divided into 3 groups based on lesion (strangulating, non-strangulating and inflammatory) the difference between these groups was not significant ($P > 0.05$). However, a significant difference was found when comparing the inflammatory or strangulating groups to the control group ($P < 0.05$), but not when comparing the non-strangulating group to the control group ($P > 0.05$). This study shows HMGB-1 is increased in horses with colic compared to normal controls. As HMGB-1 is a late-phase cytokine, it may be that more significant results will be obtained from horses with gastrointestinal disease over the course of hospitalization, rather than at admission only. Further studies are needed to determine the potential of HMGB-1 as a therapeutic target for endotoxemia in horses.

**ABSTRACT E-45**

**IN VITRO SCREENING OF FOUR NOVEL ANTI-ENDOTOXIC AGENTS USING EQUINE WHOLE BLOOD ASSAYS.** JR Bauquier, SR Bailey. University of Melbourne Faculty of Veterinary Science, Werribee, Victoria, Australia.

Endotoxemia is a common complication in horses, causing stimulation of leukocytes to produce pro-inflammatory cytokines which leads to substantial compromise to the equine patient. Due to the lack of highly effective treatments for endotoxemia in horses, there remains a need for investigation of novel therapies. The aim of this study was to determine the anti-inflammatory effect of four drugs – rolipram, azithromycin, metformin and ethyl pyruvate in equine whole blood stimulated with lipopolysaccharide (LPS). Blood was collected from 4 healthy horses for each drug tested. Drugs were added to aliquots of whole blood over a range of concentrations, after which LPS was added (E. Coli O55:B5; 1 μg/ml). Samples were then incubated at 37°C (98.6°F) for 21 hours. The anti-inflammatory effect was measured by determining tumor necrosis factor-alpha (TNF-α) levels in plasma extracted from the whole blood aliquots, using the murine L929 cell line bioassay. Inhibition of TNF-α production occurred for all drugs, with rolipram being most efficacious, having an IC50 of 1.2 μM (95% CI 0.4-3.5 μM). The IC50 values for azithromycin, metformin and ethyl pyruvate were 7.2 μM (3.0-17.6 μM), 4.5 μM (0.7-27.8 μM), and 59.9 μM (5.6-636.9 μM) respectively. Rolipram and azithromycin produced the greatest maximum inhibition at 97.2±9.0% and 98.5±10.5% respectively. The maximum inhibition caused by metformin and ethyl pyruvate were 40.9±7.5% and 26.9±7.5% respectively. These results show that rolipram, a specific phosphodiesterase inhibitor, was most effective at inhibiting TNF-α production. Further studies are needed in live horse models to determine the potential of rolipram as a therapeutic agent for endotoxemia in horses. Any potential for azithromycin as a treatment for endotoxaemia in adult horses would be limited by its contraindication due to the risk of severe colitis.

**ABSTRACT E-46**

**THE EFFECTS OF HYDROXYETHYL STARCH 6% 130/0.4 ON THROMBOELASTOGRAPHY IN HEALTHY HORSES.** A Viljoen[1], MN Saulez[1], PC Page[1], G Fosgate[2]. [1]Section of Equine Medicine, Department of Companion Animal Clinical Studies, [2]Section of Epidemiology, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, South Africa

Hydroxyethyl starch solutions (HES) restore and maintain intravascular blood volume and plasma colloid osmotic pressure (pCOP). Reported safe dosage requirements are extrapolated from human literature. Thromboelastography (TEG) has been validated for horses. The objective of the study was to determine the hemostatic and oncotic effects of HES 6% 130/0.4 (Voluven®) administered in healthy horses.

Six clinically-healthy Nooitgedacht mares were used in a randomized crossover, prospective study. Mares were assigned to 3 treatment groups and received 10, 20 and 40ml/kg Voluven® infusions with a 2-week washout period. Kaolin-activated TEG and pCOP measurements were performed before (baseline), immediately after each infusion (0h), and at 1, 6, 12, 24, 48 and 96h. TEG values: reaction time (R), clot formation time (K), maximum amplitude (MA), and angle ($\alpha$) were evaluated.

Overall mean ± SD values for 40 *vs*. 20 and 10ml/kg groups were: R = 18.5 ± 5.84min *vs*. 16.3 ± 3.41 ($P$ = .492) and 13.4 ± 2.91 ($P$ = .041); K = 4.61 ± 1.85min *vs*. 3.65 ± 0.71 ($P$ = .025) and 3.13 ± 0.74 ($P$ = .011); MA = 55.1 ± 7.43mm *vs*. 60.4 ± 4.94 ($P$ = .158) and 58.1 ± 3.99 ($P$ = .353); $\alpha$ = 39.8 ± 10.56° *vs*. 46.1 ± 6.92 ($P$ = .042) and 50.4 ± 6.60 ($P$ = .016). Overall mean ± SD pCOP values for 40 *vs*. 20 and 10ml/kg were: 24.1 ± 3.59mmHg *vs*. 21.7 ± 2.01 ($P$ = .001) and 21.0 ± 1.78 ($P$ = .001).

Compared to lower dosages, the administration of Voluven® at 40ml/kg is more likely to induce changes consistent with hypocoagulability as measured by TEG.

## ABSTRACT E-47

**OPERATOR-BASED VARIABILITY OF EQUINE THROMBOELASTOGRAPHY.** K Thane, D Bedenice, D Meola, A Pacheco. Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA.

This prospective cohort study evaluated the effect of operator-based variability on the results of thromboelastography (TEG) performed on 20 adult horses with no evidence of systemic inflammation.

Blood samples were obtained at a single time point using vacuum collection. Evaluation of physical examination and hematological parameters at the time of blood collection established that horses were free from systemic inflammation. TEG was performed in duplicate by 2 operators (2 groups of 10 horses evaluated by either O1/O2 or O1/O3) using discrete setups. TEG variables recorded for each sample included clot initiation time (R), clot formation time (K), rate of clot formation ($\alpha$), clot strength (MA), and viscoelastic/shear strength (G). All operators independently determined clinical coagulation status (normal, hypo-, or hypercoagulable) of each sample tested based on previously determined reference values for each parameter established from a population of healthy horses (range calculated as mean +/- 2 standard deviations for each variable).

For the study population, the following vital and hematological values were identified (reported as mean and range): age 8.6y (2–22y); platelet count 177,000/μL (117,000–252,000/μL); packed cell volume 35% (28–43%). Intra-operator (between duplicate samples) and inter-operator (between operators) variabilities were determined for each parameter and cohort (summarized in Table 1) using descriptive statistics and paired samples t-test (with $P < 0.05$ considered significant).

Assessment of coagulation status was well correlated between operators with agreement in 8/10 and 9/10 patient assessments for O1/O2 and O1/O3, respectively. For all TEG variables, there was no significant difference in values obtained between O1/O3; however, significantly different MA values ($p = 0.04$) and G values ($p = 0.02$) were obtained by the O1/O2 operator pair.

In conclusion, intra-operator variability of TEG ranged from 1.2-18% in this study, while inter-operator based variability minimally increased total TEG variability. Clinical coagulation assessment remained well correlated between operators.

**Abstract E-47:**

**Table 1.** Intra- and inter-operator variability of TEG values grouped by operator or operator pair. All variability values reported as: mean (standard deviation).

| | R | K | α | MA | G |
|---|---|---|---|---|---|
| Intra-operator: O1 | 9.0% (5.1%) | 14% (13%) | 9.0% (9.2%) | 2.6% (2.4%) | 6.6% (6.8%) |
| Intra-operator: O2 | 12% (9.5%) | 13% (12%) | 11% (14%) | 2.8% (2.3%) | 6.2% (4.7%) |
| Intra-operator: O3 | 13% (10%) | 18% (20%) | 10% (12%) | 1.2% (0.8%) | 2.9% (1.7%) |
| Inter-operator: O1&O2 | 13% (5.1%) | 15% (7.0%) | 12% (9.0%) | 3.2% (2.0%) | 7.3% (4.5%) |
| Inter-operator: O1&O3 | 17% (12%) | 23% (17%) | 11% (7.6%) | 2.7% (1.2%) | 6.9% (4.1%) |

## ABSTRACT E-48

**EQUINE DIARRHEA PANEL TEST RESULTS IN 308 HORSES (2007-2010).** HC Schott II[1], N Grosjean[2], S Bolin[2]. [1]Department of Large Animal Clinical Sciences; and, [2]Diagnostic Center for Population and Animal Health, College of Veterinary Medicine; Michigan State University College of Veterinary Medicine, East Lansing, MI

Establishing an etiologic diagnosis for enterocolitis in horses remains challenging. In an attempt to increase the frequency of an etiologic diagnosis in affected equids, the Diagnostic Center for Population and Animal Health at Michigan State University introduced an "equine diarrhea panel" in 2007 for testing feces and blood from horses with a clinical diagnosis of enterocolitis. The panel included a fecal culture for *Salmonella* spp, ELISA testing of feces for *Clostridium difficile* toxins A and B and *Clostridium perfringens* enterotoxin (TechLab, Blacksburg, VA), and (seasonally) PCR testing of blood and feces for *Neorickettsia risticii*. From July, 2007 through December, 2011, 322 samples were tested from 308 horses and included 166 samples submitted through the Veterinary Teaching Hospital (VTH) and 156 samples submitted from outside sources (predominantly within the upper Midwest). The most frequent positive result was for PCR testing of feces (41/156 or 26%) and blood (34/152 or 22%) for *Neorickettsia risticii* followed by positive ELISA results for *Clostridium difficile* toxins A and B (54/324 or 17%) and *Clostridium perfringens* enterotoxin (48/324 or 15%). Only 29 horses had positive fecal culture results for *Salmonella* spp during the sampling period. There were no significant differences in frequencies of positive results from samples collected from horses in the VTH as compared to those submitted from outside sources with the exception of detection of *Salmonella* spp. Of interest, 16 of the 54 samples with positive ELISA results for *Clostridium difficile* toxins A and B also had positive ELISA results for *Clostridium perfringens* enterotoxin. With regard to Potomac Horse Fever (PHF), 19 horses had positive PCR results for *Neorickettsia risticii* on both blood and feces while 14 and 18 additional horses only tested positive on blood or feces, respectively. All positive PCR results for PHF were found in samples submitted during the late spring through fall (May 13 to October 27); however, this finding is biased by the fact that testing for PHF was less commonly requested in samples submitted during the winter months. Although frequencies for positive ELISA results for clostridial toxins tended to be numerically higher in horses that also had positive PCR results for *Neorickettsia risticii* (33% for PCR+ blood samples and 34% for PCR+ fecal samples as compared to 26% for PCR- blood samples and 17% for PCR- fecal samples), Chi-square analysis did not reveal any significant differences. In conclusion, PHF remains the most frequent etiologic diagnosis for enterocolitis in horses in the upper Midwest. Further, detection of clostridial toxins in feces of one-third of horses that also had positive PCR results for PHF is a novel finding and raises the question of whether disruption of intestinal flora by *Neorickettsia risticii* infection may allow concurrent induction of toxin production by enteric *Clostridium* spp.

## ABSTRACT E-49

**OLFACTORY DETECTION OF SALMONELLA ENTERICA SEROVAR NEWPORT IN HORSE FECAL SAMPLES USING GIANT AFRICAN POUCHED RATS: A PROOF-OF-PRINCIPLE STUDY.** A Durgin[1], RD Nolen-Walston[2], N Beyene[1], A Mahoney[1], C Cox[1], B Weetjens[1], A Poling[1]. [1]Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling APOPO,

Morogoro, TZ, Africa, [2]Department of Clinical Studies - New Bolton, University of Pennsylvania, Kennett Square, PA

Nosocomial infections are a major cause of morbidity and mortality in human and veterinary patients worldwide. The rapid identification of such infections is critical in preventing such outbreaks. The hypothesis of the present study was that giant African pouched rats can distinguish rapidly and accurately between *Salmonella*-free equine fecal samples and those inoculated with *Salmonella enterica* serovar Newport. Three adult giant African pouched rats (*Cricetomys gambianus*) were trained using operant procedures to sniff sample pots within a semi-automated circular chamber containing horse fecal samples. Positive indications were defined as the rat keeping its nose in the sniffing hole for $\geq 3$ seconds. The rats were initially trained to detect *Salmonella* in culture medium, then *Salmonella*-inoculated dried horse feces, and finally *Salmonella*-inoculated fresh horse feces. Rats were trained over 25 sessions, each session consisting of 50 randomized samples, 9 *Salmonella*-positive (18%) and 41 *Salmonella*-negative.

All 3 rats were able to detect *Salmonella* accurately in all sample types. Across rats and sessions, sensitivity and specificity, respectively, were 100% and 99% when polypropylene squares served as samples; 100% and 96% when dried horse dung was used, and 100% and 96% with fresh horse manure (table 1). These results suggest that rats can discriminate between *Salmonella* and the profusion of other microbes of equine fecal flora. The use of scent-detecting pouched rats shows potential to provide highly accurate, rapid, and high-throughput detection of pathogens to screen for the presence of *Salmonella* in samples obtained from veterinary hospitals and elsewhere.

**Table 1.** Rate of false positive and false negative detections, and the probability of random positive indication by the rats using binomial distribution with a 0.5 probability of success.

| | False (-) rate | False (+) rate | Probability of results from random chance |
|---|---|---|---|
| Culture medium | 0% | 0.9% | $1.09*10^{-35}$ |
| Dried horse manure | 0.4% | 12.2% | $7.14*10^{-51}$ |
| Fresh horse manure | 0% | 11.9% | $4.01*10^{-21}$ |

## ABSTRACT E-50
## RISK FACTORS FOR ENVIRONMENTAL CONTAMINATION WITH *SALMONELLA ENTERICA* IN A VETERINARY TEACHING HOSPITAL.

BA Burgess, PS Morley. Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

*Salmonella enterica* is commonly recognized as a cause of nosocomial infections as well as zoonotic infections in veterinary teaching hospitals (VTHs). The objective of this study was to determine risk factors associated with environmental contamination of a veterinary teaching hospital with *S. enterica*.

Environmental surveillance samples were collected from February 2003 through June 2011, using a commercially available electrostatic wipe, as part of the ongoing infection control program. Sampling sites included both floor and hand contact surfaces throughout the VTH. Risk factors evaluated included hospital case load, hospital use areas, severity of disease, presence of culture positive inpatients and season. Data on risk factors of interest were collected retrospectively from the VTH medical records database. Multivariable logistic regression was used to evaluate associations between hospital risk factors and veterinary hospital environmental contamination with *S. enterica*.

During the study period, approximately 53 samples were collected monthly, for a total of 5337 environmental samples. Of the samples collected, a total of 7.9% (n = 423) were culture positive for *S. enterica* using standard culture techniques. In general, environmental samples collected in the Food Animal Hospital and floor samples were more likely to be positive.

Risk factors identified in this study will allow for the refinement of existing infection control programs as well as provide guidance to those in program development. A better understanding of the risk factors associated with environmental contamination will allow for more practical evidence based preventive measures to be implemented in veterinary hospitals experiencing epidemics of nosocomial infections with *S. enterica*.

## ABSTRACT E-51
## METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) COLONIZATION IN HORSES AT A VETERINARY TEACHING HOSPITAL.

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This study evaluated an active surveillance system for MRSA in-place at a veterinary teaching hospital to better understand the epidemiology of equine MRSA colonization and effectiveness of surveillance. MRSA screening of nasal swabs is performed in horses admitted to the Ontario Veterinary College (OVC) on admission, every 7 days in hospital, and on discharge. Data from June 1, 2009 to April 30, 2011 were analysed. Cases were classified as community-associated (CA), community onset-hospital associated (CO-HA), hospital-associated (HA) or indeterminate (IN) using standard definitions.

During the study period there were 2235 admissions (1705 different horses) and 2783 MRSA surveillance samples obtained. For admissions with a duration of at least one day (n = 1277), 728 horses (57%) were screened according to protocol, 511 (40%) were screened, but less frequently than protocol, and 38 (3%) were not screened. MRSA was isolated from 80 samples during 50 visits by 40 horses (first positive visits: CA–19; CO-HA –3; HA–17; IN–1; range 0-7 per month). Overall, 3.0% of horses were positive (monthly range 0-8.6%). The prevalence at admission (CA and CO-HA combined) was 1.7% (monthly range 0-7.1%). Three horses had MRSA infections at admission and all were also colonized. No HA clinical MRSA infections were identified. *spa* typing was available for 18 of the isolates from different horses; 16 were *spa* type t064, an ST8 strain commonly found in horses. Results for first-time positive animals were available for only 7 (18%) prior to discharge. However 11 (28%) colonized horses were later re-admitted, and 7 of these were still colonized. Within 6 months of the first positive result, 5 owners of MRSA-positive horses brought 14 additional horses to the OVC (range 1-4). Three horses from one of these owners were also colonized.

Overall, compliance with MRSA surveillance sample submissions was good. The prevalence of MRSA colonization varied throughout the study period, however the 1.7% admission prevalence is similar to previous reports from this facility. The time delay associated with sample processing hampers the effectiveness of screening as most horses have been discharged by the time results are known. However, using screening results to identify subsequent high risk cases can allow for use of enhanced infection control practices while awaiting test results as well as provide important information for assessment about the epidemiology of MRSA in the facilty. Ultimately, rapid testing is required to optimize MRSA screening in horses.

## ABSTRACT E-51B
## SEROPREVALENCE TO *NEOSPORA HUGHESI* IN HORSES WITH SUSPECTED EQUINE PROTOZOAL MYELOENCEPHALITIS.

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Neosporosis, due to *Neospora hughesi,* is one cause of equine protozoal myeloencephalitis (EPM), a progressively debilitating central nervous system disease in horses. There have been several. The incidence of EPM due to *N. hughesi* is unknown, so is its geographic occurrence. The goal of this study was to describe cases of suspected EPM that tested seropositive for *N. hughesi*.

Blood samples submitted to the Immunology Laboratory at the William R. Pritchard Veterinary Medical Teaching Hospital, University of California at Davis from 12/1/2009 to 11/30/2011 for the detection of specific antibodies to *Sarcocystis neurona* and *N. hughesi* were reviewed. The samples were accompanied by a submission form which contained information regarding the origin, signalment and neurological signs of the suspected EPM cases. Case selection included horses with neurological deficits, which tested seropositive by the NeoFluor indirect fluorescent antibody test to *N. hughesi* (titer $\geq$ 320). Concurrent titers to *S. neurona* were also evaluated.

Over the 24-month study period 7,250 submissions of suspected EPM cases were processed for antibody detection to *N. hughesi* and *S. neurona*. One-hundred and fifteen samples tested seropositive for *N. hughesi* with titers ranging from 320 to 10,240 (median 640). 54/115 (47%) *N. hughesi* seropositive horses also had antibody against *S. neurona* with titer ranging from 80 to 5,120 (median 320). In 34 dually seropositive serum samples, titers to *N. hughesi* were higher than titers to *S. neurona*. Seven serum samples had similar titers for *N. hughesi* and *S. neurona* and 13 serum samples had higher titers to *S. neurona* than to *N. hughesi*. The number of *N. hughesi s*eropositive horses ranged from 1 to 12 per month and they originated from 29 different States (TX 24 index cases; CA 18; OK 8; NY/VA 7; FL/PA/MO 5; GA/NJ 4; TN/IL/WA/NC/CT/AL/AR/ID/MT 2; MA/AZ/MN/OH/SC/IA/NV/NM/WY/WV 1). The age of seropositive horses ranged from 1 to 33 years (median 10 years) and a variety of breeds were represented including quarter horse, thoroughbred, warmblood, Arabian, draft breeds and other breeds. Most commonly reported clinical signs in *N. hughesi* seropositive index cases were ataxia (62%), weakness (38%), lameness/gait abnormality (34%) and muscle atrophy (29%).

The results of this retrospective study show that *N. hughesi* is, alone or in combination with *S. neurona*, associated with EPM cases. The wide geographic origin of *N. hughesi* seropositive horses highlights the need to test for both apicomplexan protozoal pathogens in neurologically affected horses with suspected EPM.

## ABSTRACT E-52

**A COMPARISON OF BACTERIAL COLONISATION BETWEEN TEFLON AND POLYURETHANE SHORT TERM INTRAVENOUS CATHETERS.** CW Spelta[1], RHH Tan[2], J Picard[2], B Gummow[2]. [1]Townville Vet Clinic, Townsville, Australia, [2]James Cook University, School of Veterinary Science, Townsville, Australia

The effect of catheter material on intravenous catheterisation complications in horses are unknown. This study evaluated the presence of bacterial colonisation on Teflon® and polyurethane short term intravenous catheters in healthy adult horses undergoing elective surgery.

Horses on admission for elective surgery were randomly allocated according to catheter type. Sixteen horses received Teflon® catheters and 19 received polyurethane. Aseptic catheter placement and removal was standardised, however systemic antibiotic treatment was case dependant and at the clinician's discretion. To simulate routine clinical practice, face masks were not worn during placement nor were the catheters bandaged. Catheters were maintained for 74 hours and assessed for clinical evidence of catheter site reaction, phlebitis or thrombosis twice daily.

Bacteria were cultured from 69% of Teflon® and 89% of polyurethane catheters. Multiple isolates were found in 31% of Teflon® and 42% of polyurethane catheters The Fisher exact test showed no difference between the proportion of catheters with colonisation ($P = 0.28$) or multiple isolates ($P = 0.76$). The microbes cultured were predominantly gram positive, similar to other equine and human studies. Multiple-drug resistance was seen regularly, regardless of antibiotic treatment. Despite this, no clinical evidence of phlebitis or thrombosis occurred in any horse.

It was concluded, that was no clear association between bacterial colonisation of Teflon® or polyurethane catheters ($0.9 < RR < 1.87$). The unexpected large proportion of bacterial isolates in the absence of clinical signs was also evaluated and suggests that the equine immune system plays a role in the development of septic phlebitis or thrombosis.

## ABSTRACT E-54

**PREVALENCE OF SERUM NEUTRALIZING ANTIBODIES TO EQUINE RHINITIS A AND EQUINE RHINITIS B VIRUS (ERV 1 AND ERV 2) IN SELECT REGIONS OF THE UNITED STATES.** R Keene[1], J Tuttle[1], L Mittel[2], J Morrow[3], F Andrews[4]. [1]Boehringer-Ingelheim Vetmedica, Inc. St Joseph, MO, [2]Animal Health Diagnostic Center, Cornell University Ithaca, NY, [3]Equine Diagnostic Solutions, LLC, Lexington, KY, [4]Equine Health Studies Program, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA

Equine rhinitis viruses (ERV1&2) have been associated with respiratory disease outbreaks worldwide. ERV 1&2 infections cause subclinical signs or respiratory signs including fever, nasal discharge, coughing, anorexia, pharyngitis, laryngitis and enlarged submandibular lymph nodes. Over the past decade, serologic evidence of infection has been documented in Canada, Australia, and Europe. However, recent serologic data does not exist in US horse populations. The purpose of this study was to determine seroprevalence of ERV 1&2 in different regions of the US.

Frozen serum samples were obtained from six US veterinary laboratories, Animal Health Diagnostic Center (AHDC) at Cornell University SVM, Ithaca, NY, Texas Veterinary Medical Diagnostic Laboratory Amarillo, TX, Veterinary Diagnostic Laboratory Lexington, KY, University of California at Davis Veterinary Teaching Hospital, Davis CA, Louisiana Animal Disease Diagnostic Laboratory, Equine Health Studies program, Baton Rouge, LA, and Equine Diagnostic Solutions, LLC Lexington, KY. Samples were selected from horses between 1 to 4 years of age. All samples were shipped to AHDC. Serum neutralization (SN) antibodies were determined using an established assay. SN titers $\geq$ 1:96 for ERV1&2 were considered positive.

Serum samples were evaluated in 1021 horses from regions of the US and 451 (44%) were found to have neutralizing antibodies $\geq$ 1:96 to ERV 1 and 164 (16%) were positive for ERV 2. Seroprevalence of ERV 1 was highest in samples submitted from Louisiana (49%), whereas samples from Kentucky had the lowest seroprevalence rate (34%). Seroprevalence of ERV 2 ranged from 29% in AHDC sample submissions to 15% in Louisiana samples. Specific ages were available for 554 of the samples and seroprevalence for exposure to ERV 1 appeared to increase with age, as positive titers to ERV 1 were found in yearlings (11/126 [9%]), in 2 year olds (44/116 [38%]), 3 year olds (50/163 [31%]), and 4 year olds (52/149 [35%]).

ERV has high seroprevalence in several regions of the US. SN antibodies to ERV 1 are more common than ERV 2 in the populations studied and seroprevalence appears to be age dependent. ERV 1&2 infections and their relationship to concurrent respiratory disease and respiratory pathogens in horse populations warrant further investigation.

## ABSTRACT E-55

**PREVALENCE, RECURRENCE, RISK FACTORS AND EFFECTS ON PERFORMANCE OF EPISTAXIS IN RACING THOROUGHBREDS IN THE UK: 914,849 STARTS (2001-2010).** AD Thomas[1], MJ Green[1], T Morris[1,2], N Bowen[2] GD Hallowell[1]. [1]School Of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington,UK, [2]British Horseracing Authority, London, UK

Epistaxis is a severe manifestation of exercise-induced pulmonary haemorrhage, seen in racing Thoroughbreds and other exercising horses worldwide. The objectives of this study were to report prevalence, incidence and recurrence of epistaxis, identify risk factors and report effects of epistaxis upon performance. Features of this study included the large data set, evaluation of recurrence in jump and flat racing, all in a country where race

day medication is prohibited. Cases of epistaxis were identified from all race starts (914,849) in the UK from 2001-2010 using veterinary surveillance, with additional use of stewards reports in 2010. Univariable logistic regression analyses and then multivariable mixed effect logistic regression models were built in step-wise fashion for different race types to identify risk factors. Significance was assumed as $p < 0.05$. 2076 cases of epistaxis in 1776 horses were identified. The overall prevalence of epistaxis was 0.23% and 0.32% for 2010. Prevalence was 0.13% in flat racehorses, 0.34% in hurdlers and 0.54% in steeplechasers. Incidence was 10.6 cases per 1000 horse cases per year with 2.3% of the racing population affected by epistaxis during the study period. Prevalence and incidence increased over the decade studied. Horses that raced over fences ($p < 0.001$), were competing over shorter distances (within race types; $p < 0.001$) and on firmer going ($p < 0.001$) showed increased prevalence of epistaxis. Increased risk of epistaxis was observed with age only for flat horses ($p = 0.004$). Horses with epistaxis were likely to have raced over more seasons ($p < 0.008$), but had a lower number of starts per season ($p = 0.03$) and a longer period between races ($p < 0.0001$). The number of days since the last race was higher for epistaxis performances (67 days; $p < 0.003$) when compared with controls (58 days). 13.5% of horses with epistaxis demonstrated recurrence (15.9%, 10.1% and 17.7% for flat racehorses, hurdlers and steeplechasers respectively). 71% of the recurring cases of epistaxis occurred within a year of a previous episode. Recurrence within a year was lower in steeplechase (63.7%) races when compared to flat (75.6%; $p < 0.001$) and hurdle races (76.2%; $p < 0.001$) All performance indicators which included money won, percentage of the field beaten, lengths finished behind the winner, finishing position (either won or placed in the top three) and non-completion of race demonstrated inferior race performance in those horses with epistaxis. A high percentage of horses with epistaxis did not finish (~10% flat to ~60% over jumps). These findings suggest it is the intensity of racing that affects the prevalence of epistaxis, and this supports either the capillary stress failure or locomotory impact-induced trauma theory.

## ABSTRACT E-56

**COMPARISON OF AIRWAY RESPONSE OF RECURRENT AIRWAY OBSTRUCTION AFFECTED HORSES FED STEAMED VERSUS NON-STEAMED HAY.** CA Blumerich[1], VA Buechner-Maxwell[1], WK Scarratt[1], KE Wilson[1], C Ricco[2], I Becvarova[1], J Hodgson[3], S Werre[3]. [1]Department of Large Animal Clinical Sciences, [2]Department of Small Animal Clinical Sciences, [3]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

Recurrent Airway Obstruction (RAO)-affected horses experience bronchoconstriction and airway inflammation in response to inhalation of aerosolized irritants including hay molds. Steaming hay reduces fungal content, but the effect on the antigenic potential of hay has not been investigated. We tested the hypothesis that RAO-affected horses develop less clinical disease when fed steamed versus non-steamed hay and this reduction coincides with decreased hay fungal content.

Six RAO-affected horses in clinical remission were divided into two groups and fed steamed or non-steamed hay for 10 days using a two-way cross-over design. Hay was steamed using a commercial hay-steamer [a]. Clinical assessment was performed daily. Full assessment, including upper airway endoscopy, assignment of mucous scores and measurement of maximal change in pleural pressure, was performed on days 1, 5, and 10. Bronchial fluid sampling and cytology were performed on days 1 and 10. Hay core samples were collected pre- and post-steaming and cultured to determine fungal and bacterial concentrations.

Statistical analysis was performed using SAS® and included repeated measures ANOVA, mixed model ANOVA, Wilcoxon rank-sum and Wilcoxon two sample tests. P-value $< 0.05$ was considered significant.

Horses fed non-steamed hay experienced a significant increase in clinical score ($p < 0.0001$) and a trend towards total airway neutrophilia ($p = .0834$) during the feeding period, while parameters were unchanged in horses fed steamed hay. Steaming also significantly decreased the number of fungal colony-forming-units in hay.

These results indicate that steaming reduces the RAO-affected horse's response to hay which coincides with a reduction in viable fungal content of hay.

[a]Model HG-1000, HAYGAIN hay steamer, Jiffy Steamer Equine

## ABSTRACT E-57

**COMPARISON OF AIRWAY RESPONSES IN HORSES FED ROUND BALE VERSUS SQUARE BALE HAY.** J Larson[1], Buechner-Maxwell VA[1], J Dascanio[1], S Witonsky[1], I Becvarova[1], RS Pleasant[1], F Elvinger[1], J Hodgson[2], C Cossaboom[3], C Seal[3], S Werre[2]. [1]DLACS, VMRCVM, Duckpond Drive, Blacksburg, VA, [2]DBSP, VMRCVM, Duckpond Drive, Blacksburg, VA, [3]Veterinary students, VMRCVM, Duckpond Drive, Blacksburg, VA

Feed is a major expense associated with horse ownership. Hay packaged as round bales (RB) costs approximately half that of similar square bales (SB). Recent studies indicate that feeding RB increases the risk of horses developing airway inflammation, and it is not known if this response is due to packaging, storage or hay quality differences. In this study we tested the hypothesis that there would be no difference in the respiratory health of horses fed RB as compared to horses fed SB of similar quality.

Hay harvested in mid-August from the same field and packaged as either RB or SB was fed two months later to two groups of 15 horses. RB hay was wrapped in nylon mesh, stacked and stored in an open-faced shed. SB hay was stacked and stored in an enclosed loft. At the beginning and end of a 6-week feeding period, physical and re-breathing examination, upper-airway endoscopy, trans-tracheal wash (TTW) and bronchoalveolar-lavage cytology, and TTW bacterial/fungal cultures were performed on all horses. Nutritional analysis and bacterial/fungal cultures were concurrently conducted on hay samples. Statistical analyses were completed using a mixed model ANOVA, Wilcoxon Two-sample test, Friedman's Chi-square and a Fisher's Exact T-test. Data was log transformed as needed. Analyses were performed using SAS® with $P < 0.05$ considered significant.

Horses fed RB demonstrated an increase in pharyngeal lymphoid hyperplasia ($p = 0.0143$) and percentage neutrophils ($p = 0.0078$) in the TTW samples post-feeding as compared to pre-feeding values. No additional significant differences were detected in any other measured parameters. Nutritional analysis of hay and measurements of bacterial/fungal load did not differ over time and/or between hay types.

Following a six-week exposure to RB, normal horses demonstrate subclinical evidence of upper airway inflammation as compared to horses fed SB of similar quality, suggesting that packaging contributes to this response. When eating round bales, horses bury their nostrils in hay. This behavior could result in an increased exposure to respirable particles that induce inflammation, but further studies are required to confirm this prediction.

## ABSTRACT E-58

**CARDIAC ECHOGRAPHIC EVALUATION OF EQUINE ELITE ATHLETES PRE AND POST ENDURANCE RACE.** A Giraudet[1], C Robert[2], D Maso[2], E Barrey[2]. [1]Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France, [2]INRA-UMR1313, Inserm-U902, France.

Equine echocardiography is routinely used in diseased horses or poor performers. Athletes evaluation pre and post an actual competition is less commonly performed.

Ten horses, 6 females and 4 gelding were examined the day before and immediately after an 90km endurance ride. All horses but one were 6 years old, one gelding was 11. All examined horses successfully completed the race. A partial echocardiographic examination was undertaken on every horses using a

Vivid I (GE, Healthcare). Gray scale 2 D images from right parasternal view with concurrent ECG were collected and stored in 3 full cardiac cycles cine loops for later analysis. Only long axis 4 chambers view were obtained. End systolic volume (measured at T repolarisation) and end diastolic volume (measured at Q onset) were measured and fractional ejection (FE) calculated. During the endocardium contouring process, it was noted that mitral valves closure seemed delayed in post race measurements.

Systematic measurements of mitral valves closing time compared with Q onset were performed. To adjust to heart rate variation the mitral valves closing time (MVCT) was divided by the electric systolic duration (NMVCT). Due a non parametric distribution of the calculated values (EF and NMVCT), Wilcoxon Mann Whitney Test was used to compare pre and post race measurements (P 0.05). As previously described, FE was not affected by the endurance race. NMVCT was statistically delayed after the race, confirming a post effort prolonged isovolumetric contraction.

**Table I.** Mean Pre and post race FE, normalized mitral valve closure time (NMVCT), mean mitral valve closing time delay (* : statistically significant difference).

| | Pre race FE | Post race FE | Pre NMVCT | Post NMVCT * | MVCTD |
|---|---|---|---|---|---|
| Mean | 72.48% | 77.64% | -5.3% | 6.91% | 12.24% |

## ABSTRACT E-59
## EFFECT OF EXERCISE ON PLASMA ENDOTHELIN-1 AND ANGIOTENSIN CONVERTING ENZYME IN HORSES WITH EXERCISE-INDUCED PULMONARY HEMORRHAGE.

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Horses exercising strenuously exhibit marked increase in pulmonary artery pressure, a mechanism that has been implicated in the pathophysiology of exercise-induced pulmonary hemorrhage (EIPH). Endothelin-1 (ET-1) and angiotensin converting enzyme (ACE) may act either directly or indirectly to cause pulmonary artery hypertension. Therefore, we hypothesized that horses with EIPH would exhibit higher plasma ET-1 concentration and ACE activity compared to horses without EIPH following a standardized treadmill exercise test (SET).

Eight Standardbred race horses were trained for 8 weeks before participating in a randomized, crossover, controlled trial where each horse received 0, 0.5 or 1 g/kg of sodium bicarbonate by nasogastric intubation 4 hours prior to SET performed until fatigue. Venous blood was collected prior to training, prior to SET and 15 min post-SET for ET-1 assay and measurement of ACE activity. Tracheoscopy was performed 15, 30, 60, 90, and 120 min post-SET to grade EIPH severity. Data were analyzed using analysis of variance for repeated measures. Significance level was set at $P < 0.05$.

Three horses exhibited EIPH. Plasma ET-1 concentration and ACE activity were not significantly affected by training or bicarbonate treatment. Plasma ET-1 concentration increased significantly 15 min after SET in horses without EIPH (0.99 ± 0.55 pg/ml) at which point it was significantly higher than in horses with EIPH (0.64 ± 0.34 pg/ml). ACE activity in plasma was significantly higher 15 min post-SET (69.8 ± 16.3 U/L) than before (53.8 ± 11.9 U/L) regardless of EIPH status. Blood was detected at the carina as early as 15 min post-SET with maximum EIPH score being reached at 30 min. EIPH score 120 min post-SET was significantly lower than at 30 min post-SET.

We concluded that EIPH is not associated with an elevation in plasma ET-1 concentration or ACE activity but horses without EIPH experienced an increase in plasma ET-1 15 min following strenuous exercise. Trained human athletes exhibit an increase in ET-1 that peaks 30 minutes following exercise consistent with findings in horses of this study.

## ABSTRACT E-60
## EQUINE PULMONARY CYTOKINE ENVIRONMENT AND MICROBICIDAL ACTIVITY OF MACROPHAGES IN FOALS VERSUS ADULTS.

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Pneumonia is the most common cause of morbidity and mortality in foals less than 6 months of age; however, adult horses, unless immunocompromised, are not as susceptible to such respiratory infectious agents, including *Rhodococcus equi*. We speculate that the pulmonary cytokine environment differs between the neonate and adult thereby imparting differing phenotypes to pulmonary alveolar macrophages (PAMs). This could result in differences of microbicidal activity between adult and foal monocyte-derived macrophages (MDMs) from blood and pulmonary-alveolar macrophages (PAMs) from bronchoalveolar lavage fluid (BALF). MDMs and PAMs were stimulated *in vitro* with IFNγ and subsequently infected with *R. equi* transformed with a GFP/dsRed plasmid enabling enumeration by fluorescence. Consistent with our hypothesis, MDMs and PAMs derived from foals yielded a higher bacterial load than in adults. Also, foals exhibited lower levels of peryoxynitrite production as compared to the adult. Cytokine profiles, including TNFα and IL-10 from BALF, were characterized by ELISA and yielded relative differences, particularly for TNFα. The ratio of the concentration of TNFα to IL-10 in BALF was higher in adults than in foals. Foals exhibited a lower TNFα to IL-10 ratio than adults conveying a lower pro-inflammatory cytokine milieu. In addition, the IL-10 concentration was higher in the supernatant of *R. equi* infected MDMs and PAMs. Taken together, these critical differences indicate that foal macrophages are more susceptible to infection with *Rhodococcus equi* and support the notion that the cytokine milieu impacts the microbicidal response of the macrophage.

## ABSTRACT E-61
## EQUINE MONOCYTE-DERIVED DENDRITIC CELLS STIMULATE PROLIFERATION AND FOXP3 EXPRESSION IN AUTOLOGOUS, REACTIVE T CELLS.

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Dendritic cells (DCs) represent a heterogeneous population of innate immune cells specialized in immune surveillance, antigen presentation, and initiation of the adaptive immune response. DCs are uniquely capable of stimulating naïve T cells and can generate a variety of effector responses, anergy, or even tolerance. Therefore, DCs are an essential component of a successful immune response, and a detailed understanding of the interactions between DCs and T cells is relevant to many areas of immunology. We have developed a method of isolating and co-culturing relatively pure populations of equine monocyte-derived DCs with autologous, CFSE-stained peripheral blood T cells. The magnitude and nature of the DC-induced T cell response was monitored using multi-color flow cytometry to measure T cell proliferation, surface marker expression, and cytokine production.

We discovered that equine monocyte-derived DCs, but not macrophages, are able to potently induce proliferation of autologous, reactive T cells in the absence of foreign antigen ($p < 0.002$). This process, termed the autologous mixed leukocyte reaction (AMLR), is a defining feature of DCs not yet described in cells from a domestic animal species. The equine AMLR is contact-dependent ($p < 0.005$), MHC class II-dependent ($p < 0.02$), and primarily involves CD3+, CD4+, CD8- T cells. A subset of the proliferating T cells expresses the regulatory T

cell (Treg) transcription factor FoxP3. Many of these DC-stimulated, FoxP3+ T cells can produce effector cytokines, indicating that FoxP3 expression in the horse, in contrast to the mouse, is regulated similarly to FoxP3 expression in man. However, the FoxP3+ population is enriched for IL-10-competent cells, which suggests that equine monocyte-derived DCs promote the generation of Tregs *in vitro*.

Our study describes the stimulatory capacity of equine monocyte-derived DCs and permits the analysis of autologous, reactive T cells that may play an important role in immune regulation. Furthermore, we anticipate that future antigen-specific analyses of the interactions between DCs and T cells in this manner will enhance our knowledge of the equine immune system and promote the development of improved immunotherapeutic strategies.

## ABSTRACT E-62

**ARE BLOOD EQUINE NEUTROPHILS INHERENTLY RESISTANT TO GLUCOCORTICOIDS?** G Hirsch, A Lavoie-Lamoureux, J-P Lavoie. Université de Montréal, St-Hyacinthe, QC/CA

Human neutrophils are considered poorly responsive to glucocorticoids (GC). The reported increase in survival caused by GC on this cell population partly supports this assertion. In heaves-affected horses, the persistent airway neutrophilia after GC administration may be explained by this process. However, it was recently shown that dexamethasone exerts potent anti-inflammatory effects on equine peripheral blood neutrophils through both genomic and non-genomic pathways.

…Because objective support for the corticoresistance of neutrophils is lacking, we assess the effects of three glucocorticoids on apoptosis of equine blood neutrophils and compare their transrepression effects on neutrophils and mononuclear cells. To determine if these effects are species specific, data obtained from horses were compared with those of human neutrophils and mononuclear cells.

Peripheral blood neutrophils and mononuclear cells were isolated from 6 healthy horses. To assess apoptosis, neutrophils were incubated 20h with hydrocortisone, prednisolone or dexamethasone ($10^{-8}$M to $10^{-6}$M). Quantification of Annexin V-APC, and 7-AAD binding was performed by flow cytometry. To evaluate transrepression effects, neutrophils and mononuclear cells were incubated 5h with or without LPS (100ng/mL) alone or combined with GC. IL-8, IL-1β and TNF-α mRNA expression was quantified by qPCR. Transrepression effects of dexamethasone on human neutrophils and mononuclear cells were also studied.

Stimulation with all three GC resulted in a significant increase in equine neutrophil survival. They also significantly down-regulated the LPS-induced mRNA expression of the three genes of both equine and human neutrophils and mononuclear cells. These effects operate in a dose-dependent manner and dexamethasone appears to be the most potent drug.

These results indicate that glucocorticoids increase survival in equine neutrophils but that these cells appear at least as responsive as mononuclear cells to glucocorticoids.

## ABSTRACT E-63

**HEMATOPOIESIS IN THE EQUINE FETAL LIVER SUGGESTS IMMUNE PREPAREDNESS.** JM Battista, T Stokol, MJB Felippe. College of Veterinary Medicine, Cornell University, Ithaca, NY

Our laboratory studies the development of the immune system of the horse. Though B lymphocyte development is one of the best-studied hematopoietic pathways, little is known about its origin in the equine fetus, although it can produce antigen-specific immunoglobulins around 200 days of gestation. Studies in other species show that hematopoietic stem cells (HSC) generated in the aorta-gonad-mesonephros region and yolk sac seed the fetal liver, which becomes the main hematopoietic organ and supports the development of B lymphocytes during fetal life.

| | $V_H$ | $J_H$ | Vλ | Jλ |
|---|---|---|---|---|
| **Percent Nucleotide Identity with Equine Genomic Sequence** | | | | |
| Fetal Liver | 98.5 | 97.9 | 99.3 | 98.6 |
| Adult Bone Marrow | 88.4 | 94.7 | 88.0 | 93.6 |

The purpose of our study was to learn how B cell hematopoiesis and immunoglobulin production develop in the equine fetus during gestation. Our hypothesis was that hematopoiesis occurs in the liver at early gestation with the generation of B cells with limited immunoglobulin diversity. Equine fetal liver cells were harvested around 100 days of gestation, and the presence of hematopoietic stem cells and leukocytes were investigated at the molecular and cellular levels.

The mRNA expression of molecules and transcription factors involved in early lineage-specific hematopoietic differentiation including c-KIT, CD34, IL7R, CXCL12, IRF8, PU.1, PAX5, NOTCH1, GATA1, CEBP/A were detected using RT-PCR. Immunohistochemistry and flow cytometric analysis indicated that approximately 5% of the cells were positive for the HSC marker CD34, whereas 1-5% of cells stained positive for T lymphocyte markers (CD2, CD3, CD4, CD5, CD8) and B lymphocyte markers (CD19, IgM), and 3-15% of cells expressed myeloid markers (CD11b, CD172a). To characterize the pre-immune B cell repertoire, heavy and lambda immunoglobulin light chain V(D)J segments were cloned, sequenced, and analyzed. The fetal liver sequences had similar V(D)J gene segment usage and complementarity determining region 3 (CDR3) amino acid length, but had a higher degree of nucleotide identity with germline sequences compared to those isolated from adult horse bone marrow.

Our study demonstrates, for the first time, active B cell hematopoiesis in the equine fetal liver around 100 days of gestation, with a diverse, albeit in lower magnitude, pre-immune immunoglobulin repertoire in comparison to the adult horse. The immunoglobulin diversity develops in an essentially sterile environment, appears more extensive than other reported species, and may have implications in equine neonatal immune-competence and response to vaccines.

## ABSTRACT E-64

**VERTEBRAL OSTEOMYELITIS AND DISKOSPONDYLITIS IN FOALS.** MC Coleman[1], MK Chaffin[1], J Griffin[1], WV Corapi[1], TE Norman[1], NM Slovis[2], AL Johnson[3], KG Magdesian[4], CC Clark[5]. [1]Texas A&M College of Veterinary Medicine, College Station, TX, [2]Hagyard Equine Medical Center, Lexington, KY, [3]University of Pennsylvania New Bolton Center, Kennett Square, PA, [4]University of California School of Veterinary Medicine, Davis, CA, [5]Peterson and Smith Equine Hospital, Ocala, FL

There are several cases reports in the literature of vertebral osteomyelitis and/or diskospondylitis in the foal; however, to the authors' knowledge there are no large studies evaluating the clinical features and outcome of this disease in foals.

The objective of this study was to describe the signalment, clinical signs, results of clinicopathologic testing, results of diagnostic imaging (radiography, CT, ultrasound) and necropsy examination, results of microbiologic testing, location of lesions, treatment, and outcome of foals less than 1 year of age affected with vertebral osteomyelitis and/or diskospondylitis.

Data from 15 foals, from 5 different referral centers were retrospectively evaluated. Foals less than 1 year of age with radiographic and/or necropsy findings consistent with osteolysis were included in the study. Foals ranged in age from 2 weeks to 4 months. There were 11 males and 4 females with a variety of

breeds represented. The most common clinical signs included fever, tachycardia, tachypnea, anorexia, and lethargy. Spinal ataxia and recumbency were noted in 1/2 and 1/5 of foals, respectively. Clinicopathologic features were variable, with an elevated white blood cell count in half of the cases. Lesions in the cervical vertebral body were most common. Evidence of a septic process of another body system was noted in less than half of the cases. Seven foals survived to discharge.

In conclusion, vertebral osteomyelitis and diskospondylitis are uncommonly reported in foals. Clinical signs and clinicopathologic features are variable. Prognosis is guarded for affected foals.

## ABSTRACT E-65

**VERTEBRAL OSTEOMYELITIS AND DISKOSPONDYLITIS IN ADULT HORSES.** MC Coleman[1], MK Chaffin[1], J Griffin[1], WV Corapi[1], TE Norman[1], AL Johnson[2], KG Magdesian[3]. [1]Texas A&M College of Veterinary Medicine, College Station, TX, [2]University of Pennsylvania New Bolton Center, Kennett Square, PA, [3]University of California School of Veterinary Medicine, Davis, CA

Vertebral osteomyelitis is uncommonly reported in adult horses. While there are several case reports of vertebral osteomyelitis and/or diskospondylitis in the literature, to the authors' knowledge there are no large studies evaluating the clinical features and outcome of this disease in adult horses.

The objective of this study was to describe the signalment, clinical signs, results of clinicopathologic testing, results of diagnostic imaging (radiography, CT, ultrasound) and necropsy examination, results of microbiologic testing, location of lesions, treatment, and outcome of adult horses affected with vertebral osteomyelitis and/or diskospondylitis.

Data from 19 adult horses, from 3 different referral centers were retrospectively evaluated. Cases with radiographic and/or necropsy findings consistent with osteolysis were included in the study. Cases consisted of 11 males and 8 females, ranging in age from 2-24 years. A variety of breeds were represented. The most common clinical signs included stiffness and poor performance. Neurologic deficits were noted in 1/3 of cases, all characterized by symmetric ataxia in all four limbs. Clinicopathologic features were variable, with a normal white blood cell count in 80% of the cases. Lesions in the cervical vertebral body were most common. Lysis of adjacent vertebral endplates were noted in 2/3 of cases. Thirteen horses survived to discharge, with 7 returning to the previous level of performance.

In conclusion, vertebral osteomyelitis and diskospondylitis are uncommonly reported in horses. Clinical signs and clinicopathologic features are variable. Prognosis is guarded to fair.

## ABSTRACT E-66

**CLASSIFICATION AND PREDICTIVE FACTORS OF SEIZURES IN HORSES.** VA Lacombe[1,2], M Mayes[2], S Mosseri[3], SM Reed[4], TH Ou[5]. [1]Department of Veterinary Clinical Sciences, [2]College of Pharmacy, The Ohio State University, Columbus, OH, [3]The Equine Clinic, Peyrolles, France, [4]Rood & Riddle Equine Hospital, Lexington, KY, [5]The University of Michigan College of Pharmacy, Ann Arbor, MI

Although many studies have been performed to classify seizures in humans and small animals, similar epidemiologic study is lacking in horses. The purpose of this study was to characterize seizures based on their type in 104 horses presented for seizure disorders at The Ohio State University Veterinary Medical Center in a retrospective case series.

Seizures were classified based on ictal phenomenology and seizure type, according to most recent accepted definitions in both human and small animal epileptology. History, clinical and neurological observations, diagnostic investigations (e.g., electroencephalograms, cerebrospinal fluid analysis and computed tomography imaging of the head) and postmortem examinations, when available, were recorded for univariate and multivariate logistic regression analyses.

Seizures were categorized as primary generalized in 24% of horses, simple and complex partial without secondary generalization in 45% of cases, secondary generalized in 20% of cases and unclassified in 11% of cases. Status epilepticus was recorded in 2% of cases. Seizure type was not associated with etiology. For a horse with recurrent seizures (i.e., epilepsy), the odds of having partial seizures was higher ($P < 0.05$) compared to a similar horse with generalized seizures in the multivariate logistic regression analysis.

This study provided a comprehensive classification of seizures by type in a referral-based equine population. Similar to human and small animal classification, the majority of the seizures described were partial seizures with or without secondary generalization. Predictive factors of epilepsy in horses were similar to those reported in other species and may assist the clinician with the early diagnosis of epilepsy.

## ABSTRACT E-67

**BRAINSTEM AUDITORY EVOKED RESPONSE IN FOALS UP TO 6 MONTHS OLD: REFERENCE VALUES AND EFFECT OF AGE, RATE OF ACOUSTIC STIMULATION AND NEUROLOGICAL DEFICITS.** L Lecoq, M Gains, L Blond, J Parent. Faculté de médecine vétérinaire Université de Montréal, Saint-Hyacinthe, QC, Canada

Age and rate of acoustic stimulation affect peak latencies in brainstem auditory evoked responses (BAER) in human. Those effects are unknown in foals. The goals of this study were to 1) establish references values for BAER in foals using 3 different stimulation protocols, 2) evaluate the effects of age and rate of stimulation on BAER traces in foals up to 6 months old, and 3) compare the data with BAER obtained from foals with CNS disorders.

Thirty nine foals neurologically normal and 16 foals with neurological diseases. BAER were recorded using 3 different protocols of stimulation (11.33 Hz/70 dBNHL; 11.33 Hz/90 dBNHL; 90 Hz/70 dBNHL).

No effects of age were observed in normal foals ($p > 0.005$). No significant differences were observed for latencies and interpeak latencies (IPL) when neurological foals were compared to normal foals ($p > 0.05$). Increasing the stimulation rate did not improve detection of CNS disorders. All neurologically abnormal foals had latencies and IPL within reference values but 78.6% had an asymmetry in their traces, reflecting a difference in conduction time between the left and right size of the brainstem.

We provide reference values of BAER for foals up to 6 months using 3 different protocols. Further investigations are needed to conclude on the use of an increased rate of acoustic stimulation in foals. Most importantly, most foals with neurological deficits had also an abnormal BAER. This proves BAER is useful is the early diagnosis of neurological disorders in foals.

## ABSTRACT E-68

**VALIDATION AND RELIABILITY OF ORTHOGONAL ULTRASONOGRAPHIC PROJECTION DIMENSIONS OF THE KIDNEY IN THE HORSE.** JL Habershon-Butcher, IM Bowen, GD Hallowell. School Of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington,UK

The aims of this study were to evaluate a novel translumbar (TL) ultrasonographic method for assessment of renal dimensions, to establish a normal reference range and to assess reliability of renal dimensions obtained using this new approach when compared with a conventional transabdominal (TA) ultrasonographic technique and measurements obtained at postmortem. Six Thoroughbred or TB cross horses, weighing 513±49Kg, were examined prior to slaughter. Both kidneys were imaged in short and long axis using the TL and conventional TA projections. Both cranial and caudal poles of the right kidney were assessed. A minimum of twelve ultrasonographic cineloops were obtained and stored for later analysis from both the left and right kidneys. The overall length, width and depth were determined, as were the thickness of the cortex, medulla and

**Abstract E-68:**

**Table 1.** demonstrating normal reference ranges (Ref ranges; mean±2SD) and mean±SD for maximal renal dimensions obtained in the harvested kidneys (PME) and from ultrasonograms using the novel technique (Translumbar; TL) and from conventional transabdominal (TA) ultrasonography. Maximal left renal dimensions were obtained in the 16th ICS and those for the right in the 15th ICS.

| | PME | | Translumbar (TL) | | Transabdominal (TA) | |
|---|---|---|---|---|---|---|
| Parameter (cm) | Ref ranges | Mean±SD | Ref ranges | Mean±SD | Ref ranges | Mean±SD |
| Left renal length | 13.4-18.6 | 16.0±1.3 | 10.6-17.8 | 14.2±1.8 | 10.5-19.7 | 15.1±2.3 |
| Left renal width | 11.6-14 | 12.8±0.6 | 10.4-14 | 12.2±0.9 | 11.4-13.4 | 12.4±0.5 |
| Left renal depth | 4.2-8.2 | 6.2±1.0 | 6.0-9.2 | 7.6±0.8 | 4.6-8.6 | 6.6±1.0 |
| Right renal length | 13.1-19.1 | 16.1±1.5 | 14.7-19.9 | 17.3±1.3 | 13.9-18.7 | 16.3±1.2 |
| Right renal width | 11-15 | 13.0±1.0 | 13.0-17.8 | 15.4±1.2 | 11.3-16.9 | 14.1±1.4 |

pelvis. The measurements were repeated in cadaveric material. All measurements were undertaken by two observers to assess reproducibility and measured on three separate occasions by one observer to evaluate measurement repeatability. Measurements were compared using Student's T-test, ANOVA and post-hoc Bonferoni and coefficient of variation and reliability was assessed using repeated measures ANOVA and intra-class correlation coefficients (ICC's).

Both kidneys were consistently identified by both methods in the 15-17th intercostal spaces (ICS) and paralumbar fossa with maximal dimensions obtained for the left kidney in the 16th ICS and for the right kidney in the 15th ICS. Image quality was good to excellent for both techniques. There was no difference between dimensions obtained by TA or TL projections and good correlation between ultrasound dimensions and post-mortem measurements (Table 1; ICC > 0.8). Excellent repeatability and reproducibility (ICC > 0.80) was obtained for all measurements. Reliability was better for larger structures. The TL technique is easily performed and produces reliable dimensions that may assist with the diagnosis of renal disease in horses.

## ABSTRACT E-69

## EFFECTS OF ORAL SODIUM BICARBONATE ADMINISTRATION ON URINE NET BASE EXCRETION AND STRONG ION DIFFERENCE IN STANDARDBRED HORSES.

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It is widely recognized within the horse-racing industry that accurate tests for the pre-race administration of alkalinizing agents need to be developed. Measurement of urinary net base excretion (NBE) or net acid excretion (NAE, where NAE = -NBE) provides a more sensitive and specific method for evaluating acid-base balance than venous blood pH, bicarbonate, base excess or urinary pH. We hypothesized that urinary NBE and strong ion difference (SID) have utility as confirmatory tests for the race day administration of bicarbonate or other alkalinizing agents in trained Standardbreds.

Four female Standardbreds (2 to 6 years of age) were fed a typical training diet (DCAD ≈ 200 mEq/kg DM) and trained 5 times a week for 6 weeks using a high-speed treadmill. Sodium bicarbonate was administered by nasal intubation at 0, 0.5, and 1.0 g/kg BW in 3 L of distilled water in a randomized crossover design 4 hours before horses underwent a simulated race protocol (SRP) on a high-speed treadmill. Urine samples were collected via a Foley catheter immediately before treatment (time = 0), at 2, 4, and 8 hours after treatment, and at 30 minutes and 90 minutes after the end of the SRP. Urine NBE and ammonium concentration ($[NH_4^+]$) were determined using an automatic titrator (Schott Instruments, Mainz Germany) and urine pH was determined using a glass electrode. Urinary $[Na^+]$, $[K^+]$, and $[Cl^-]$ were measured using appropriate dilutions on a Nova 4 analyzer (Nova Biomedical, Waltham MA) and urinary SID was calculated as $SID = [Na^+] + [K^+] - [Cl^-]$. Data was analyzed using repeated measures analysis of variance and $P < 0.05$ was significant using Bonferroni corrected post-tests.

Oral sodium bicarbonate administration induced marked, dose-dependent, and statistically significant changes in urine NBE, pH, and SID. Urine NBE was increased in horses administered sodium bicarbonate, relative to untreated controls, at all time-points after time = 2 hours except 90 minutes after the end of the SRP in horses treated with 0.5 g/kg. Urine pH was increased in both groups of horses administered sodium bicarbonate, relative to untreated controls, at 30 minutes after the end of the SRP and at time = 8 hours. Urine SID was increased in horses administered sodium bicarbonate, relative to untreated controls, at all time-points after time = 2 hours except at time = 4 hours in horses treated with 0.5 g/kg. Urine pH was related to NBE and urine $[NH_4^+]$ such that: $pH = a + \log_{10}(NBE + [NH_4^+])$. We conclude that the pre-race administration of oral sodium bicarbonate induced marked, persistent, and statistically significant changes in urine NBE, urine pH, and SID. Our findings suggest that urine NBE and SID may have value as confirmatory tests for the race day administration of alkalinizing agents in trained Standardbreds.

## ABSTRACT E-70

## EFFECTS OF MELOXICAM AND PHENYLBUTAZONE ON RENAL RESPONSES TO FUROSEMIDE, DOBUTAMINE AND EXERCISE IN HEALTHY HORSES.

SL Raidal, KJ Hughes, AL Charman, S Nielson, GK Noble. School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia

Non-steroidal anti-inflammatory drugs are widely used in equine practice, exerting their therapeutic effect by decreasing cyclooxygenase (COX) activity and thereby limiting production of pro-inflammatory prostaglandins. However, prostanoids mediate a number of protective mechanisms which are particularly important for gastrointestinal and renal homeostasis. It has been hypothesised that drugs which selectively target COX-2, the induced isoform of this enzyme, may be safer analgesic and anti-inflammatory agents than non-selective COX inhibitors. The present study was undertaken to compare the effects of meloxicam, a COX-2 selective non-steroidal anti-inflammatory drug (NSAID), with those of the non-selective drug phenylbutazone on renal responses of healthy adult horses to furosemide, dobutamine and sub-maximal exercise.

Ureteral catheterisation was used to determine urine flow and to collect samples for determination of exogenous creatinine clearance during three hours of confinement following administration of placebo, phenylbutazone (PBZ, 4.4 mg/kg PO) or meloxicam (MEL, 0.6 mg/kg PO) to nine lightbreed mares. Treatment order was randomised and a Latin square design utilised such that each horse received all treatments. Thereafter, the effect of NSAID treatment on responses of these horses to the subsequent administration of furosemide (1 mg/kg IV), dobutamine (5 μg/min/kg IV for 30 minutes) and submaximal exercise were assessed over a similar time period in three consecutive studies.

Consistent with previous studies, NSAID administration had no significant effect on baseline renal function of horses. Prior treatment with PBZ or MEL attenuated increases in urine flow

associated with the administration of furosemide or following exercise. Dobutamine infusion was associated with transient increases in mean arterial pressure and urine output in all treatment groups. Although COX-2 expression has not been evaluated in the equine kidney, the results of this study suggest that selective COX-2 inhibitors, such as MEL, are likely to have similar effects on renal prostaglandin synthesis as non-selective agents, and so pose similar risks for renal adverse effects in susceptible animals.

## ABSTRACT E-71

**EFFECT OF BUTORPHANOL ON THERMAL NOCICEPTIVE THRESHOLD IN HEALTHY PONY FOALS.**

KT McGowan[1], JR Elfenbein[1,2], SA Robertson[1], LC Sanchez[1]. [1]University of Florida College of Veterinary Medicine, Gainesville, FL, [2]Texas A&M University College of Veterinary Medicine and Biomedical Sciences, College Station, TX

Analgesia is an important component of foal medical care, and no objective data currently exist regarding the analgesic efficacy of opioids in foals. The study objective was to evaluate the somatic anti-nociceptive effects of butorphanol in healthy foals. The hypothesis was that thermal nociceptive threshold would increase following intravenous butorphanol but not intravenous saline administration in neonatal and older pony foals.

Seven healthy neonatal pony foals, 1-2 weeks of age, and 11 healthy older pony foals, 4-8 weeks of age were evaluated. Some foals were used during both age periods. Interventions, which included saline (0.5 mL IV), butorphanol (0.05 mg/kg) and butorphanol (0.1mg/kg) were administered in a blinded, randomized cross-over design with at least 2 days between interventions. Each dose was adjusted with saline to a total volume of 0.5 mL. Response variables included thermal (TT) nociceptive threshold, skin temperature (ST) and behavior score. Each was recorded prior to and 15, 30, 45, 60, 90, 120, 150, and 180 minutes following treatment.

A significant ($P < 0.05$) increase in thermal threshold, relative to time 0, was noted following butorphanol (0.01mg/kg) administration at times 15-60 and 120-150 minutes in neonates and at times 15-90 and 150 minutes in older foals. Significant time effects were not noted within other treatments in either age group. No significant time or treatment effects were apparent for skin temperature. Significant time, but not treatment, effects were evident for behavior score in both age periods.

Butorphanol shows analgesic potential in both neonatal and weanling age foals.

## ABSTRACT E-72

**SYSTEMIC AND ANTI-NOCICEPTIVE EFFECTS OF PROLONGED CONTINUOUS RATE INFUSIONS OF ANALGESIC COMBINATIONS IN HEALTHY HORSES.**

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Analgesic combinations are used to treat horses with severe signs of pain. The purpose of this study was to determine the effects of prolonged constant rate infusions (CRI) of lidocaine (L), butorphanol (B), and ketamine (K) alone and in combination on gastrointestinal transit, behavior, and thermal nociceptive threshold in healthy horses.

Eight healthy adult horses, each equipped with a gastric cannula for purposes of other studies were used. Interventions were saline, L, K, B, LK, LB, BK, and LBK as an intravenous CRI for 96 hours in a randomized cross-over design with investigators blinded to treatment. Drugs were mixed or diluted in saline; CRI rate was 0.15mL/kg/hr (Table). Two-hundred plastic beads were administered intragastrically by nasogastric tube immediately prior to the bolus. Feces were collected every 2 hours, weighed, and beads manually retrieved. Behavior was scored every 2 hours, vital parameters every 6 hours, and wireless thermal threshold every 12 hours for 96 hours.

Four of 64 trials (3 LBK, 1 BK) were discontinued early due to mild colic. There were no apparent differences between groups in vital parameters or thermal threshold. Transit time was delayed for LB and LBK with a corresponding decrease in fecal weight.

Analgesic combinations cause a delay in gastrointestinal transit in healthy horses without substantially affecting somatic nociception at the doses studied.

| Drug | Bolus (15 minutes) | CRI |
|---|---|---|
| Saline | 60 mL | 0.15 mL/kg/hr |
| Lidocaine | 1.3 mg/kg | 3 mg/kg/hr |
| Butorphanol | 0.018 mg/kg | 0.013 mg/kg/hr |
| Ketamine | 0.55 mg/kg | 0.5 mg/kg/hr |

## ABSTRACT E-73

**FIROCOXIB LOADING DOSE TRIAL IN HORSES.**

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The objective of this study was to determine if a single loading dose, three times the label dose of firocoxib oral paste, followed by nine maintenance doses at the current label dose achieves and maintains near steady state concentrations. Six healthy, adult mares received 0.3 mg/kg of firocoxib on Day 0, followed by 0.1 mg/kg administered at 24 hours on Day 1, and at 24 hour intervals Day 2 through Day 9, for a total of 10 doses. Blood samples were collected at 0 (prior to treatment), 0.25, 0.5, 1, 2, 4, 6, and 12 hours post treatment on Day 0, prior to treatment on Day 1, and at the same times as Day 0. Blood samples were also collected prior to each treatment on Days 2-9 and at 0.25, 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours post treatment on Day 9. Samples were centrifuged and plasma stored frozen until analyzed by a validated HPLC-MS method.

After the initial loading dose of 0.3 mg/kg (3X label dose), the average maximum concentration was 199 ng/mL. After the first maintenance dose of 0.1 mg/kg the maximum average concentration was 171 ng/mL. At steady state (after the last dose) the maximum average concentration was 183 ng/mL. This indicates near steady state concentrations are achieved after the first dose. The maximum and minimum plasma concentrations (Cmax and Cmin) at steady state ranged from 110-243 ng/mL and 50-168 ng/mL, respectively. There was no indication of unexpected accumulation. The average AUC (0-tau) was 150 ng/mL*days. Plasma concentrations decreased in a monoexponential manner after the last dose and the average half life was 1.74 days.

## ABSTRACT E-74

**SAFETY AND PHARMACOKINETICS OF TOPICAL 1% DICLOFENAC SODIUM CREAM IN HEALTHY NEONATAL FOALS.**

S Barnett[1], D Sellon[1], M Hines[1], D Neelis[1], R Mealey[1], H Knych[2], R Keene[3]. [1]Washington State University, Pullman, WA, [2]University of California-Davis, Davis, CA, [3]Boehringer Ingelheim, St. Joseph, MO

This study evaluated the biochemical, renal, and gastrointestinal effects of topical 1% diclofenac sodium cream (Surpass®) in healthy neonatal foals.

A randomized, blinded, controlled, clinical trial was performed on 12 healthy Arabian or part-Arabian foals that were 2-14 days of age and weighed 27-54 kilograms on day 1 of the study. Cream (7.3 mg [1.3 cm strip] of diclofenac or equivalent volume of Cetaphil® hand moisturizer) was applied to a clipped area over the left tibiotarsal joint twice daily for 7 days. Serum chemistry, CBC, urinalysis, urine gamma-glutamyltransferase:urine creatinine (uGGT:uCr), renal and right dorsal colon ultrasound,

and gastric endoscopy were evaluated on the first and last days of study. Foals were monitored daily and showed no signs of systemic illness during the study. Venous blood was obtained at specific time points for pharmacokinetic analysis. Data were compared using 2-sample T-test or ANOVA as appropriate. No adverse events related to diclofenac treatment occurred. No statistically significant differences ($p \leq 0.05$) between groups were observed. Changes in uGGT:uCr ratios, which were considered the most sensitive parameters, were not different between groups ($p = 0.93$). Skin and hair growth were normal at the application site throughout the study and at 2-6 months following the study. Plasma concentrations of diclofenac were low but detectable in treatment foals throughout the study.

Diclofenac sodium applied to clipped skin of healthy neonatal foals at 7.3 mg twice daily for 7 days was a safe dosing regimen. Evaluation of efficacy in reducing pain and inflammation in the equine neonate is warranted.

## ABSTRACT E-75

**PHARMACOKINETICS OF GALLIUM MALTOLATE IN HEALTHY AND *LAWSONIA INTRACELLULARIS*-INFECTED RABBITS.** F Sampieri[1], J Alcorn[2], AL Allen[1], CR Clark[1], FA Vannucci[3], N Pusterla[4], KR Ball[1], PM Dowling[1], J Thompson[5], LR Bernstein[6], CJ Gebhart[3], DL Hamilton[1]. [1]Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, [2]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada, [3]College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, [4]School of Veterinary Medicine, University of California, Davis, CA, [5]Canadian Light Source, Saskatoon, SK, Canada, [6]Terrametrix, Menlo Park, CA, USA

We hypothesized that gallium maltolate (GaM) may prove effective as a novel antimicrobial agent for weanling foals affected by equine proliferative enteropathy (EPE), caused by *Lawsonia intracellularis* (LI). This is one of a series of experiments where, in a rabbit infection model of EPE, we addressed pharmacokinetics (PK) and pharmacodynamics characterization of GaM. Since gallium may interfere with iron uptake pathways, we compared PK and intestinal tissue (IT) concentrations of elemental gallium **[Ga]** and iron **[Fe]** after oral administration of GaM to LI-infected and uninfected rabbits. The Animal Research Ethics Board of the University of Saskatchewan approved these studies.

Twelve, 8 to 9 wk old pathogen-free New Zealand White does, 2 to 2.5 kg, were divided into 2 groups of 6 animals: uninfected group (control) and LI-infected group (EPE). Both groups were treated once intragastrically with 50 mg/kg GaM. EPE rabbits were treated 7 days post infection. Serial blood samples were taken from an indwelling auricular arterial catheter, from time 0 to 216h (9 days) post-treatment (PT). At 9 days PT, rabbits were sacrificed and IT samples were collected from intestinal sections where EPE lesions are characteristically identified. Tissues were frozen at -20°C or fixed in 10% phosphate buffered formalin. Presence or absence of EPE in rabbits was confirmed via detection of LI-specific antibodies using immuno-peroxidase monolayer assay, quantitative PCR on feces and immunohistochemistry on IT. Elemental **[**Ga] and [Fe**]** were determined in IT and blood **(**[Ga] only) using inductively coupled plasma - mass spectrometry. A non-compartmental approach was used for PK parameter estimation. Statistical comparison (alpha always set at 5%) of PK parameters in the 2 groups was performed with Student t-test; and **[**Ga] and [Fe] comparison in IT with one-way ANOVA.

The log-linear terminal phase rate constant for gallium was lower ($p = 0.03$) in EPE-rabbits ($0.0171 \pm 0.0028$ (SD) $h^{-1}$ in controls and $0.0116 \pm 0.004$ (SD) $h^{-1}$ in EPE-rabbits, consistent with the longer terminal gallium half-life (t½) observed in EPE-rabbits ($59.4 \pm 24.0$h vs $39.4 \pm 10.8$h in controls). No other PK parameter estimates were significantly different between groups. In controls and EPE-rabbits respectively, $C_{max}$ was 0.5± 0.214 vs 0.59± 0.416 ug/mL; $t_{max}$ was $1.75 \pm 0.41$ vs $0.9 \pm 0.37$ h; oral clearance was $2.5 \pm 0.9$ vs $3.04 \pm 1.14$ ul/h. At 9 days PT [Ga] and [Fe] in IT were higher ($p < 0.0001$) in controls. Our results suggest that administration of GaM every 48h, is an appropriate dosing interval for multi-dose studies in rabbits, since EPE increases GaM t½. Furthermore, GaM-dosed EPE rabbits not only have reduced [Ga] in IT, but also reduced [Fe], despite its consistent availability in the diet.

## ABSTRACT E-76

**EFFICACY OF GALLIUM MALTOLATE AGAINST *LAWSONIA INTRACELLULARIS* INFECTION IN A RABBIT MODEL.** F Sampieri[1], A.L Allen[1], J Alcorn[2], CR Clark[1], FA Vannucci[3], N Pusterla[4], KR Ball[1], PM Dowling[1], J Thompson[5], LR Bernstein[6], CJ Gebhart[3], D.L Hamilton[1]. [1]Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, [2]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada, [3]College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, [4]School of Veterinary Medicine, University of California, Davis, CA, [5]Canadian Light Source, Saskatoon, SK, Canada; [6]Terrametrix, Menlo Park CA, USA

Rabbits act as a time-compressed infection model for weanling foals affected by equine proliferative enteropathy (EPE), due to *Lawsonia intracellularis* (LI). As gallium maltolate's (GaM) antimicrobial efficacy against LI is difficult to evaluate *in vitro*, we administered GaM to LI-infected rabbits (GAL-LI). Also, we compared GaM efficacy with untreated LI-infected controls (CON-LI) and doxycycline-treated LI-infected positive controls (DOXY-LI), as doxycycline is the leading treatment in clinical cases. The Animal Research Ethics Board of the University of Saskatchewan approved this study.

Twenty-four, 5 to 6 wk old, pathogen-free New Zealand White does, 1.2 to1.5 kg, were infected intragastrically with LI (approx. $2.5 \times 10^8$ LI/rabbit) and then assigned to 3 groups (8 animals each): untreated (CON-LI), medium-only treatment, q 24h, orally; GaM-treated (GAL-LI) 50 mg/kg q 48h orally; and doxycycline-treated (DOXY-LI) 5 mg/kg q 24h, orally. Treatment started 7 days post-infection for each group. Feces were collected daily from each group and blood was collected from each doe once weekly for serology. A week from the first treatment, all rabbits were sacrificed and gastrointestinal tissues (GIT) were collected from intestinal tracts characteristically affected by EPE. Samples were frozen at -20°C, or immediately fixed in 10% phosphate buffered formalin. In all groups, EPE was confirmed via detection of LI-specific antibodies using immuno-peroxidase monolayer assay, quantitative PCR on feces and immuno-histochemistry (IHC) on GIT. Inductively coupled plasma-mass spectrometry was used to quantify gallium [Ga] and iron [Fe] concentrations in GIT and blood ([Ga] only). Statistical analysis (alpha always set at 5%) was performed using a contingency table and Chi-square test for the number of lesions detected via IHC in each GIT section of the 3 groups; a one-way ANOVA was used to compare [Ga] and [Fe] in GIT.

EPE lesions were typically stained via IHC in mid- and end-jejunum and cecum, but no difference due to treatment was detected in the said GIT (p values 0.35; 0.77; 0.74 respectively). None of the treatments caused a significant difference in fecal shedding detected by qPCR ($p = 0.64$). Also, multiple GaM treatment led to higher [Ga] and [Fe] in the cecum ($p = 0.0064$ and $< 0.0001$, respectively), compared to other GIT. Multiple doses of GaM did not cause any adverse effect in rabbits; however, our results show that GaM is not more efficacious than the leading clinical treatment (doxycycline) in the EPE rabbit infection model.

## ABSTRACT E-77

**PHARMACOKINETICS OF INTRAVENOUS CONSTANT RATE INFUIONS OF CEFTIOFUR SODIUM IN THE ADULT HORSE.** S Khalfan, S Edwards SL Raidal. School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia

Ceftiofur is a time dependent bacteriocidal antimicrobial. As such, plasma concentrations should be maintained above the mean inhibitory concentration (MIC) of target organism(s) for greater than 80% of the inter-dosing interval. Many Gram negative pathogens have MICs approaching or exceeding 1 – 2 µg/mL, concentrations which are unlikely to be maintained *in vivo* by the use of intermittent dosing. In human patients, β-lactam

antimicrobials are often administered as a continuous rate infusion (CRI) to ensure that plasma concentrations are maintained at high levels for the duration of treatment. For hospitalised equine patients, a CRI is readily implemented and may offer a therapeutic and economic advantages over bolus treatments.

Ceftiofur sodium (285 μg/kg/h) was administered by CRI to six healthy thoroughbred / standardbred mares (age 3 to 9 years, body weight 488 to 630 kg) for 48 hours following a 3.2 mg/kg IV loading dose. Plasma samples were collected for determination of desfuroylceftiofur concentrations prior to loading and at 1, 5, 10, 15, 30 and 60 min following commencement of treatment, and then at regular intervals throughout the duration of infusion. Plasma samples were also collected after 48 hours of treatment and at 1, 2, 3, 4, 8, 12 and 18 h following completion of CRI. Synovial fluid samples were collected from intercarpal and radiocarpal joints after 48 h CRI, and at 2, 8 and 18h following completion of treatment.

CRIs were well tolerated by all horses. Appetite, demeanour, faecal output and consistency were unaffected by treatment. No changes were observed in haematological or serum chemistry parameters post-infusion, when compared with values obtained prior to infusion. Peak plasma defuroylceftiofur concentrations (28.2 ± 8.3 μg/mL, mean ± sd; range 20.4 to 41.7 μg/mL) were observed 1 min following administration of the loading dose. Thereafter plasma concentrations plateaued by 3 to 4 hours after commencement of CRI and remained constant at 6.3 ± 1.3 μg/mL. Synovial fluid concentrations at 48 h were 2.0 ± 0.8 μg/mL. The total amount of ceftiofur administered to each horse was approximately 46% of the total dose administered via bolus dosing at 10 mg/kg q 12h.

## ABSTRACT E-78
## USE OF COMBINATION OF TAZAROTENE CREAM AND IMIQUIMOD 5% CREAM IN THE TREATMENT OF EQUINE SARCOIDS: A 20 CASE RETROSPECTIVE STUDY.

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Sarcoids are the most common cutaneous neoplasms of horses. Despite the numerous types of treatments that have been developed, none is universally effective, and the rate of recurrence of surgical excision is usually high. Successful management has been achieved with the use of radiotherapy and chemotherapy, and more recently with the use of electrochemotherapy.

However biosafety constraints restrict their use to qualified equine structures. Topical treatments which could be used in ambulatory practice at the owners place could offer a valuable alternative to these treatments. Imiquimod, an imidazoquinolamine, is an immune response modifier with antitumoral and antiviral activity successfully used in humans in the treatment of cutaneous tumours such as melanomas, carcinomas and actinic keratosis. In equine medicine, a 5% imiquimod cream has been tested successfully in the treatment of equine sarcoids and aural plaque. Tazarotene normalizes the behaviour of the keratinocytes, which is used for its keratolytic activity on verrucous (warty) form of equine sarcoids. The aim of this study was to confirm the efficacy of 5% imiquimod in treatment of equine sarcoid, and to evaluate the benefit of a prior treatment with a 0.1% tazarotene cream. 24 cases were treated from June 2007 to June 2010. 20 cases totalizing 83 tumours (T0 to T3 according to Owen's classification) met the criteria for inclusion in the study. The four remaining cases were excluded because of lack of treatment compliance. Animals were also required to have complete medical records and a minimum of 12 months follow-up. Two large tumours (one T3 and one T4) were subjected to prior debulking. Tazarotene 0.1% cream was applied daily for two weeks prior to imiquimod 5% cream which initial prescription duration was every other day for 12 weeks and pursued until complete resolution of tumours if necessary. All tumours responded well with a complete response rate of 63% at 12 weeks, 87% at 16 weeks and up to 100% at 21 weeks. There was no influence of the tumour size on the duration of treatment ($p = 0.153$). 35% of lesions resulted in persistent alopecia after treatment but alopecia was significantly correlated ($p = 0.0001$) with keratinization (verrucous sarcoids) which resulted in pretreatment alopecia. In 53% of tumours hair regrowth was observed while leucotrichia was observed in 12% of treated tumours. The major side effect was inflammation and pain at the treatment site especially in thin skin regions. This was controlled in most cases with NSAIDs administration but was also the cause of some treatments lack of compliance. This study provides additional evidence of the effectiveness of imiquimod on superficial sarcoids and suggests that the combination of tazarotene and imiquimod topical treatments may improve the results compared to previous studies.

## ABSTRACT E-79
## REDUCED CARDIAC OUTPUT IN HORSES ANESTHETIZED AFTER DANTROLENE ADMINISTRATION.

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Dantrolene may prevent post-anesthetic myopathy in horses. This study examined the effect of dantrolene on cardiac output (CO) and other variables in healthy horses under general anesthesia, and on the duration and quality of anesthetic recovery.

In a randomized cross-over trial, six horses were administered dantrolene (6 mg/kg in 2L of water) or water (2L) via nasogastric tube one hour before commencing 90 minutes of isoflurane anesthesia. CO was measured via lithium dilution technique seven times during anesthesia concurrently with analysis of plasma potassium concentration. Anesthetic recovery was scored by two anesthesiologists blinded to treatment groups. Serum creatine kinase (CK) was measured before and 4, 8 and 12 hours after anesthesia. Data were analyzed by repeated measures ANOVA ($p < 0.05$).

Dantrolene caused significantly lower CO (L/min) at the 2nd (30.5±2.8 vs. 24.4±2.1), 3rd (28.1±1.3 vs. 20.9±3.0) and 4th (27.9±0.9 vs. 20.9±2.5) measurements after which time difficulty obtaining valid measurements in dantrolene-treated horses suggested further decline. Plasma potassium (mEq/L) was significantly higher after dantrolene at the 5th (4.5±0.3 vs. 5.2±0.3), 6th (4.5±0.2 vs. 5.3±0.5), and 7th (4.5±0.2 vs. 5.9±0.7) measurements. Cardiac arrhythmias necessitated premature recovery of two dantrolene-treated horses. Recovery time was not different between treatments but quality score was superior after dantrolene. Serum CK (U/L) was significantly lower 4 hours (2873±1721 vs. 325±79) and 8 hours (3159±2073 vs. 351±88) after anesthesia with dantrolene.

In conclusion, dantrolene reduces muscle damage in horses undergoing general anesthesia without inhibiting anesthetic recovery, but decreases cardiac output and can precipitate hyperkalemia and arrhythmias.

## ABSTRACT E-80
## RESPONSE OF ACTIVATED EQUINE CHONDROCYTES FROM CARPAL AND HOCK JOINTS TO AN AVOCADO/SOYBEAN UNSAPONIFIABLES, GLUCOSAMINE, CHONDROITIN SULFATE, PENTOSAN POLYSULFATE, AND N-ACETYL GLUCOSAMINE COMBINATION.

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The pathogenesis of osteoarthritis (OA) has been attributed to up-regulation of pro-inflammatory molecule expression in chondrocytes and other joint tissues. These molecules include prostaglandin E2, cytokines, and chemokines. Expression of these genes is regulated by the transcription factor nuclear factor-kappa B (NF-B). Inhibition of their up-regulation is a major objective for the management of OA. While OA affects articular cartilage in different joints, little is known whether the inflammatory response of chondrocytes vary from different sites. In the present study, we compared the response of chondrocytes isolated from carpal and hock cartilage to cytokine stimulation. We also evaluated whether these stimulated chondrocytes would display similar responses to the mixture of avocado/soybean unsaponifiables

(ASU), glucosamine (GLU), chondroitin sulfate (CS), pentosan polysulfate (PPS), and N-acetyl glucosamine (NG). These compounds have been shown to provide anti-inflammatory and chondroprotective effects *in vivo* and *in vitro*. ASU, GLU, and CS are also been used for the management of equine OA.

Chondrocytes ($5x10^5$ cells/well) were pre-incubated for 24 hours with: (i) control media alone or (ii) the combination of ASU (NMX1000®, 8.3μg/mL) + GLU (FCHG49®, 11μg/mL) + CS (TRH122®, 20μg/mL) + [PPS + NG] (AUPEN5000TM, PPS: 125μg/mL, NG: 200μg/mL). Cells were activated with interleukin-1β (IL-1β; 10ng/mL) for 1 hour. Total RNA was isolated and real-time PCR used to quantify IL-1β, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), cyclooxyengase-2 (COX-2), and interleukin-8 (IL-8) gene expression. Intracellular localization of NF-κB was determined by immunohistochemistry. Statistical analysis was run using one-way ANOVA and the Tukey post-hoc test with significance set at $P < 0.05$.

Carpal and hock chondrocytes showed similar responses to cytokine activation resulting in increased pro-inflammatory gene expression. Cytokine activation in carpal and hock-derived chondrocytes was significantly ($P < 0.001$) and similarly inhibited by ASU + GLU + CS + [PPS + NG]. Inflammatory gene expression suppression was accompanied by NF-κB translocation inhibition.

These findings support the utility of the ASU + GLU + CS + [PPS + NG] combination for the management of fore- or hind-joint inflammation in horses and other species. (Research supported by Nutramax Laboratories, Inc.)

## ABSTRACT E-81
## LAMINAR ANTIOXIDANT GENE EXPRESSION IN EXPERIMENTALLY-INDUCED EQUINE LAMINITIS: A COMPARISON OF TWO MODELS.

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Oxidative stress reportedly plays an important role in sepsis-induced organ dysfunction/failure in many species. While oxidative events are also likely to occur in laminae in sepsis-associated laminitis, we have recently reported minimal evidence of end-stage oxidative tissue injury in models of sepsis-related laminitis. A robust antioxidant response to oxidative events might underlie the lack of oxidative tissue modification observed in the digital laminae of horses with experimentally-induced laminitis. The laminar antioxidant response to experimentally-induced equine laminitis has not been fully characterized, and this response has not been directly compared between the black walnut extract (BWE) and enteral carbohydrate overload (CHO) models of laminitis. Antioxidant enzymes likely to be present in laminar tissue include superoxide dismutase 1 (SOD1)/cytosolic SOD, SOD2/mitochondrial SOD, SOD3/extracellular SOD, glutathione peroxidase 1 (GPX1, cytosolic), and GPX4 (membrane-associated). We hypothesized that these genes would be expressed at higher concentrations in the laminae of laminitic animals compared with that of control animals. Archived digital laminar tissue collected at various time points from adult horses subjected to BWE- (n = 15) and CHO- (n = 20) induced laminitis were used for this study. Laminar antioxidant gene expression was evaluated at both the transcript (RT-qPCR) and protein (Western immunoblot) levels. In the CHO model, no change in mRNA concentration of GPX1 (p = 0.91), GPX4 (p = 0.43), SOD1 (p = 0.56), or SOD3 (p = 0.29) was detected at the developmental or OG1 time points when compared with controls. The concentration of SOD2 mRNA, however, was significantly elevated at the OG1 time point when compared with controls (p = 0.0019). In the BWE model, laminar mRNA concentrations of GPX4 (p = 0.018) and SOD2 (p = 0.0004) were significantly increased at the OG1 time point when compared with samples from both control and developmental time points. Concentrations of SOD1 and SOD3 mRNA were significantly lower at the OG1 time point compared with the developmental time point in this model (p = 0.0075 and p = 0.021, respectively). The protein concentration of SOD2 within digital laminar tissue was increased at the developmental and OG1 time points in both models; this was the only gene evaluated that displayed regulation at the protein level. The results of this study suggest that up-regulation of antioxidant genes within the digital laminae may play a role in mitigating the extent of oxidative tissue modification that occurs in the setting of experimentally-induced laminitis; future studies including assessments of non-enzymatic antioxidants and total reductive capacity of laminar tissue would further our understanding of the laminar antioxidant response.

## ABSTRACT E-82
## PLASMA ATRIAL NATRIURETIC PEPTIDE CONCENTRATIONS AT REST IN HORSES WITH VARIOUS HEART DISEASES.

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Atrial natriuretic peptide (ANP) is physiologically expressed in the heart and mainly released from atrial tissue after myocardial stretch. Moreover, the expression in ventricular myocytes can be enhanced by cardiac diseases. We hypothesized that horses with heart diseases affecting left atrial (LA) dimensions and/or function or left ventricular (LV) dimensions and/or function have higher plasma ANP concentrations ($C_{ANP}$) than healthy controls and that $C_{ANP}$ can be used diagnostically to identify horses with cardiac disease associated with alterations in chamber size and function.

Blood samples were collected from 61 horses with heart diseases (age 14 ± 6 years, body weight 542 ± 102 kg [mean ± SD]) and from 15 healthy horses (10 ± 4 years, 589 ± 47 kg). The horses were assigned to 4 groups based on history, physical examination and echocardiographic examination: (1) healthy controls, (2) horses with heart disease but normal chamber size and function, (3) horses with heart disease, abnormal LA size and/or function, and normal LV, and (4) horses with heart disease with both abnormal LA and LV size and/or function. $C_{ANP}$ was measured by a commercially available radio-immunoassay (ANP(1-28) (human, bovine, porcine) RIA kit (S-2011), Peninsula Laboratories, San Carlos CA, USA). Log ($C_{ANP}$) was compared (a) between controls and all horses with disease using Student's t-test and (b) between all 4 groups using one-way ANOVA with Holm-Sidak post-hoc test for multiple comparisons. ROC analyses were used to describe test characteristics and to identify appropriate cut-off values.

$C_{ANP}$ was higher in horses with heart disease (103.0 [13.5-488.0] pg/mL, median [range]) compared to healthy controls (65.0 [22.0-92.0] pg/mL; p = 0.01). When considering all 4 groups, the difference was only significant between control horses and group 4 (137.0 [13.5-488.0] pg/mL). At a cut-off value of 95.5 pg/mL, $C_{ANP}$ had a sensitivity of 57% (95% CI: 44-70%) and a specificity of 100% (95% CI: 78-100%) for diagnosing heart disease in horses. However, the test did not allow discrimination between groups 2, 3 and 4.

In conclusion, $C_{ANP}$ increases with heart diseases affecting chamber dimensions and/or function. Using the RIA applied in this study, $C_{ANP}$ can be used as a biomarker to identify horses with heart disease with high specificity but low sensitivity. The diagnostic value of this biomarker is compromised by the large overlap in $C_{ANP}$ between healthy horses and horses with heart disease.

## E-82B
## IMMUNOLOGIC AND PHYSIOLOGIC RESPONSE TO VACCINATION AGAINST ANGIOTENSIN II IN ADULT HORSES.

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Hypertension is documented in equine diseases including metabolic syndrome, laminitis and chronic renal failure. Currently, effective treatments for hypertension in the horse are limited. The purpose of this study was to investigate the immunologic response of normotensive adult horses to vaccination with AngQb, a modified angiotensin II structure linked to virus-like particles, and determine its effect on arterial blood pressure.

Six adult horses were vaccinated intramuscularly with 900 μg of AngQb on three different occasions, one month apart. Serum samples for anti-angiotensin II antibody titer determination were

collected prior to vaccination, and weekly through week 16, and on week 20. Indirect blood pressure was measured prior to vaccination, and every 2 weeks through week 16 and weeks 20 and 24.

Mean antibody titer was determined by ELISA and peaked at 21,400 ± 19,380 on week 6 and was 9,986 ± 10,792 at week 20. Data were evaluated statistically by repeated measures ANOVA with Dunnett's multiple comparison post hoc analysis and Pearson correlation. Systolic arterial pressure increased at weeks 8 and 20 compared to baseline ($P = 0.008$). Neither diastolic nor mean arterial pressure were significantly changed over time, however there was a significant negative correlation of mean antibody titer with both mean ($R = -0.24$; $P = 0.03$) and diastolic ($R = -0.22$; $P = 0.05$) arterial pressure. While a robust immunologic response to AngQb vaccination was demonstrated, additional studies are warranted to evaluate arterial blood pressure responses in hypertensive horses.

**ABSTRACT E-83**
**HYPERTENSIVE CARDIOMYOPATHY IN HORSES.** C Navas de Solis[1], J Slack[1], V.B Reef[1]. [1]New Bolton Center, University of Pennsylvania, Kennett Square, Pennsylvania

The objective was to describe the clinical presentation, physical examination, prognosis, echocardiographic and pathologic findings of horses with hypertensive cardiomyopathy. Records from cases presented between 1995 and 2011 diagnosed with cardiac hypertrophy and systemic hypertension (systolic and mean non invasive blood pressure > 144 and 116 mmHg respectively) were studied retrospectively.

Five horses met the inclusion criteria (0.26% of horses evaluated for cardiac disease). There were 3 geldings and 2 mares of multiple breeds with a mean±SD age of 18±4 years (range 13-24). The main diagnoses were chronic laminitis in 3 cases and chronic renal failure in two. Persistent tachycardia, hypertension, chronic laminitis or a combination of these prompted the cardiac evaluations. Non invasive blood pressure [systolic/diastolic (mean)] was 212±36 mmHg [range 183-261 mmHg] / 169±38 mmHg [range 100-190 mmHg] (171±41 mmHg [range 126-222 mmHg]). No arrhythmias were reported. All horses had increased relative wall thickness 0.94±0.29 [range 0.57-1.26]. The interventricular septum was thickened in all horses at end diastole and in 4 horses at peak systole. The left ventricular free wall was thickened and the internal diameter reduced at end diastole and peak systole in three horses. All horses were euthanized due to grave prognosis of the primary disease. All three horses that underwent necropsy had macroscopic cardiac hypertrophy and 2 had microscopic cardiovascular changes.

Hypertensive cardiomyopathy is rare and was associated with chronic pain or renal disease. Information about the reversibility, the importance of early detection of cardiomyopathy in hypertensive horses and its long-term sequelae is needed.

**ABSTRACT E-84**
**OXIDATIVE METABOLISM AND *IN VITRO* IMMUNE RESPONSE OF HORSES SUPPLEMENTED WITH VITAMIN COMPLEX ADE.** RW Weigel, M Mirian Souza, FN Fernandes, WR Della Libera, AMMP SucupiraM.C.A.. University of São Paulo, Brazil

Horses working at explosive exercise are subjected to oxidative stress. Animals unable to balance the production of reactive oxygen species (ROS) and antioxidant reaction can lose performance and have longer recover time. The aim of this study was to evaluate oxidative metabolism and *in vitro* immune response of polo players equines treated or not with parenteral ADE vitamin complex.

Twenty horses, crossbred, 7.0 (± 1.7) yrs of age, trained for equestrian polo matches were used. They were randomly distributed in two groups. Control group received 10 mL of physiologic saline solution, IM, and the Treated group received 1ml/50kgBW IM of the supplement containing vitamins A (27 000 000 UI / 100mL), $D_3$ (8 000 000 UI/100mL) e E (8 000 mg/100mL). The horses were at rest 60 days before the game. The research began with an application 30 and 15 days before the start of training. At the first training blood samples were collected before the game (M0), 15 minutes (M1), 90 minutes (M2) and 3 hours (M3) after the only chukker. Plasma was assayed for malondialdehyde (MDA). Superoxide dismutase (SOD) concentration was determinate in red blood cells. Reduced glutathione (GSH) and burst-phagocytosis were determined in total blood. The burst-phagocytosis was performed by flow cytometry. Treatments were compared by ANOVA (Tukey test) and Pearson coefficient was determinated between variables using Statistical Software MINITAB®.

There were no differences in the concentrations of MDA, SOD, GSH and burst-phagocytosis between Control and Treated groups ($P > 0.05$). Only the SOD concentration and phagocytic intensity were negatively correlationated ($r = -0.302$ and $P = 0.1$). In this study parenteral ADE supplementation didn't make a difference in the oxidative metabolism and *in vitro* immune response in horses trained for polo matches.

**ABSTRACT E-85**
**ASSESSMENT OF VITAMIN E LEVELS IN A POPULATION OF HEALTHY ADULT HORSES.** K Vander Werf, EG Davis, C Blevins. Kansas State University, Manhattan, KS

Equine Motor Neuron Disease (EMND) is associated with chronic vitamin E deficiency resulting in neuromuscular weakness, hyperhidrosis, preferred recumbency, and muscle fasciculation. Among 7 clinical cases presented within the past 3 years, 57% had adequate serum vitamin E levels (ref. range: 2.0-4.0 μg/mL).

Serum tocopherol levels in 50 healthy, adult horses maintained on dry lots and 50 horses maintained on pasture for at least 1 year were analyzed at a commercial laboratory. Variables examined included vital parameters, environment (dry lot vs pasture), duration in environment, and concentrate supplementation. Horses were excluded if in the current environment for less than 1 year.

Vitamin E levels of horses maintained on pasture (5.44 ± 2.26 μg/mL) and those maintained on dry lot (mean: 3.99 ± 2.84 μg/mL) were significantly different ($p = 0.0003$). Additionally, there was a significant positive correlation between age and tocopherol level ($r = 0.30$, $p = 0.0026$). Geldings maintained on dry lots had significantly lower tocopherol levels than mares maintained in the same environment or geldings and mares on pasture ($p = 0.03$). There was no significant correlation between breed, brand of pelleted feed, or duration in current environment and serum vitamin E levels.

Horses maintained on dry lots should be monitored for signs of EMND and should have vitamin E levels measured on a regular basis. Geldings appear to be at risk of low vitamin E levels when maintained in dry lot environments. Supplementation with natural vitamin E is recommended for horses with levels less than 2.0 μg/mL.

# SMALL ANIMAL – ENDOCRINOLOGY

**ABSTRACT EN-1**
**RESTORATION OF EUTHYROIDISM IN MEDICALLY TREATED HYPERTHYROID CATS WITH IATROGENIC HYPOTHYROIDISM (IH) IMPROVES RENAL FUNCTION.** TL Williams, J Elliott, HM Syme. Royal Veterinary College, London, UK

IH is reported to increase the incidence of azotemia in hyperthyroid cats following treatment. IH is also associated with a decreased heart rate (HR), packed cell volume (PCV) and plasma alkaline phosphatase activity (ALP). Hypothyroidism reduces glomerular filtration rate (GFR) in other species, and treatment of hypothyroidism in dogs is reported to increase GFR. There-

fore, it could be postulated that adjustment of anti-thyroid medication in cats with IH to achieve euthyroidism would also improve GFR, and hence reduce plasma creatinine concentration and the incidence of azotemia. This study aimed to compare renal function in cats with IH before and after dose adjustment of anti-thyroid medication (methimazole or carbimazole) to achieve euthyroidism.

Medically treated hyperthyroid cats with documented IH (plasma total thyroxine concentration (TT4) < 10 nmol/l in combination with plasma cTSH > 0.15 ng/ml) were prospectively recruited into the study between July 2009 and March 2011. Dose adjustments were made to anti-thyroid medication every four weeks until euthyroidism was restored (TT4 10-40 nmol/l). If restoration of euthyroidism was achieved more than eight months after documentation of IH, the cat was excluded from further analysis. Body weight (BW), body condition score (BCS), systolic blood pressure (SBP), PCV, HR and plasma concentrations of urea, creatinine, cholesterol, total calcium and plasma activities of alanine aminotransferase (ALT) and ALP were recorded at baseline and once euthyroidism was achieved. Continuous data are presented as median [25$^{th}$, 75$^{th}$ percentile]. Clinicopathological parameters were compared at baseline and following restoration of euthyroidism using the Wilcoxon signed rank test. Statistical significance was defined as $P < 0.05$.

Twelve cats were eligible for inclusion. Baseline TT4 and cTSH of the cats were < 4.0 [< 4.0, 6.1] nmol/l and 3.45 [0.48, 5.93] ng/ml respectively. At baseline, 9/12 cats were azotemic (plasma creatinine concentration > 2 mg/dl). TT4 following dose adjustment was 24.9 [15.4, 26.6] nmol/l. Restoration of euthyroidism resulted in a significant decrease in plasma creatinine concentration (2.50 [1.92, 3.16] mg/dl vs. 2.07 [1.42, 2.66] mg/dl; $P = 0.005$), and azotemia resolved in 3/9 cats. Restoration of euthyroidism also resulted in a significant increase in plasma ALP (20.8 [15.8, 30.8] U/l vs. 31.5 [23.2, 45.7] U/l; $P = 0.004$) and HR (186 [174, 204] beats per minute (bpm) vs. 214 [182, 225] bpm; $P = 0.034$), and a significant decrease in BW (3.91 [3.36, 4.15] kg vs. 3.70 [3.23, 4.16] kg; $P = 0.026$). No significant changes in BCS ($P = 0.603$), SBP ($P = 0.875$), PCV ($P = 0.329$), plasma concentrations of urea ($P = 0.239$), cholesterol ($P = 0.530$), total calcium ($P = 0.182$) or plasma ALT activity ($P = 0.388$) were observed following restoration of euthyroidism.

Restoration of euthyroidism in cats with IH improves renal function, evaluated using plasma creatinine concentration, and decreases the incidence of azotemia, although changes in BW could influence plasma creatinine concentrations. Measurement of GFR in cats with IH before and after dose adjustment is warranted.

## ABSTRACT EN-2

## THYROID SCINTIGRAPHY FINDINGS IN 917 CATS WITH HYPERTHYROIDISM.

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Thyroid scintigraphy provides valuable information regarding both thyroid anatomy and physiology and plays an integral role in the diagnosis, staging, and management of feline thyroid disease. Recently, Harvey et al (Scintigraphic findings in 120 hyperthyroid cats. JFMS 2009;11: 96) reported that nearly 1 of 5 hyperthyroid cats had multiple areas of increased radionuclide uptake (IRU) visible on thyroid imaging, commonly with intrathoracic tissue that could not be palpated. Many of those cats, however, were referred for persistent or recurrent hyperthyroidism so this may not represent what is seen in general practice. In this study, we performed thyroid imaging on 917 consecutive hyperthyroid cats that were referred for radioiodine therapy between January and December 2009. Scintigraphy was performed as part of our staging protocol in which thyroid volume is estimated for $^{131}$I dose estimation (Vet Radiol Ultrasound 1996;27:141).

Of the 917 cats, 594 (65%) had bilateral thyroid lobe uptake in the "expected" neck location, whereas 279 (30%) had unilateral thyroid uptake. The remaining 44 cats (4.8%) had multiple areas of IRU or areas of ectopic tissue not in the neck area. Of the 917 cats, areas of IRU ranged from 1-5 (median 2), with 39 (4.2%) cats having > 3 (Table 1). Areas of IRU were located in the neck in 906 (98.8%), thoracic inlet in 105 (11.5%), and in the thorax in 44 (4.8%); 22 cats (2.4%) had IRU in all 3 locations (neck, thoracic inlet, thorax). The estimated size of the thyroid tumor(s) in the 917 cats were small (< 2.5 cm$^3$) in 517 (56%), medium-sized (2.5-5 cm$^3$) in 243 (26%), and large (> 5 cm$^3$) in 166 (18%). Cats with large thyroid tumors had a higher pretreatment median serum T4 value (20.6 μg/dl) than did cats with medium-sized (13 μg/dl) or small masses (6.4 μg/dl). Cats with large tumor volumes had been hyperthyroid for longer (median, 12 mos) than were cats with small or medium-sized tumors (1 mo). Of the 157 cats with large thyroid masses, 14 were suspected of having thyroid carcinoma, with an overall incidence of 1.7%. Ectopic thyroid tissue was diagnosed in 30 (3.3%) cats; of these, large tumor size was found in only 6 cats (20%).

In conclusion, results of this study confirm that multiple areas of hyperfunctional thyroid tissue do develop in hyperthyroid cats, but at an incidence much lower than previously reported. Ectopic thyroid tissue occurs in ˜3-4% of hyperthyroid cats, many with only mild to moderate hyperthyroidism. Thyroid carcinoma develops in ˜1.5% of cats, but these cats characteristically have huge goiters, very high serum T4 values, and long-standing hyperthyroidism.

**Table 1.** Total number and locations of areas of increased radionuclide uptake (IRU).

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Total number of cats | 286 | 592 | 28 | 7 | 4 |
| No. of cats with areas of IRU in neck | 276 | 534 | 28 | 6 | 4 |
| No. of cats with areas of IRU in thoracic inlet | 27 | 58 | 12 | 5 | 3 |
| No. of cats with areas of IRU in thorax | 2 | 12 | 17 | 6 | 4 |

## ABSTRACT EN-3

## AMINO ACID, IODINE, SELENIUM, AND COAT COLOR STATUS AMONG HYPERTHYROID, SIAMESE, AND AGE-MATCHED CONTROL CATS.

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Hyperthyroidism is common among older cats, but its pathogenesis remains poorly understood. Siamese and Himalayan cats have a reduced risk of hyperthyroidism than other cat breeds. Tyrosine is the amino acid precursor for thyroxine and melanin. Earlier studies reported tyrosine as a limiting amino acid in some cat foods resulting in poor coat melanin production in dark-coated cats. Because Siamese and Himalayan cats have unique tyrosine metabolism responsible for a pointed coat color, this study evaluated the relationship between tyrosine status and coat color in hyperthyroid cats. In addition, key cofactors in thyroid hormone metabolism (iodine and selenium) were evaluated as co-dependent variables. The objective of this study was to determine if tyrosine, phenylalanine, iodine, or selenium levels are altered in hyperthyroid cats compared to normal cats and if light or pointed coat color is protective with greater tyrosine availability due to lowered use for melanin production.

**Table 1.** Plasma tyrosine, plasma phenylalanine, serum iodine, and serum selenium levels in hyperthyroid cats compared to control cats (range; median value).

| | Tyrosine (nmol/mL) | Phenylalanine (nmol/mL) | Iodine (ppm) | Selenium (ppm) |
|---|---|---|---|---|
| Hyperthyroid (n = 12) | 56-88; median 61 | 61-91; median 82 | 0.13-0.46; median 0.19 | 0.37-0.55; median 0.48 |
| Control (n = 15) | 38-95; median 67 | 62-126; median 93 | 0.08-0.30; median 0.17 | 0.36-0.60; median 0.47 |

Twenty-seven client-owned cats with (n = 12) and without (n = 15) hyperthyroidism were studied. Coat color (9 white or pointed; 18 dark), breed, and diet history were recorded. Whole blood was collected for CBC, serum chemistry, total thyroxine (TT4), serum iodine, serum selenium, and plasma amino acid determination in fasted cats. A mixed model ANOVA with cat and group included as class variables was used to evaluate the relationship between group study factors (significance = $P \leq 0.05$).

Chemistry and CBC values were similar among groups except for TT4 levels. Tyrosine, phenylalanine, iodine, and selenium levels were not significantly different among light or dark cats or cats with or without hyperthyroidism. Altered tyrosine metabolism associated with coat color does not explain the reduced risk of hyperthyroidism in pointed or light coat colored cats.

## ABSTRACT EN-4

**EFFECT OF THE STAGE OF CHRONIC KIDNEY DISEASE ON SERUM TOTAL THYROXINE IN CATS.** AB Vieira, MCN Castro, M Salomão, LC Gershony, AMB Soares, AMR Ferreira. College of Veterinary Medicine, Universidade Federal Fluminense, Rio de Janeiro, Brazil

Non-thyroidal illnesses, such as chronic kidney disease, are one of the factors that can affect total thyroxine and hinder the diagnosis of hyperthyroidism in cats. Cats with chronic kidney disease are currently staged according to the degree of azotemia based on the IRIS (International Renal Interest Society) guidelines. The purpose of the present study was to determine the influence of chronic kidney disease stage on serum total thyroxine (TT4) in cats.

Total thyroxine was measured by chemiluminescent immunometric assay in the serum of 32 healthy cats and 33 cats with various degrees of chronic kidney disease. Nineteen cats were classified as IRIS stage 2, nine cats as IRIS stage 3 and four cats as IRIS stage 4.

Cats with chronic kidney disease had significantly lower ($p < 0.0001$) mean serum TT4 concentrations than clinically healthy cats. Mean serum TT4 concentration decreased 34.73% in the IRIS stage 2 group, 41.67% in the IRIS stage 3 group and 55.58% in the IRIS stage 4 group when compared to the control group. Even though mean TT4 concentrations decreased with the increase of azotemia in the CKD groups, values were only significantly different when compared to cats in the control group.

Results obtained in the present study suggest that any stage of chronic kidney disease can lead to the suppression of total thyroxine concentration in cats.

## ABSTRACT EN-5

**ASPART INSULIN CONSTANT RATE INFUSION FOR TREATMENT OF DOGS WITH DIABETIC KETOACIDOSIS.** ES Walsh, KJ Drobatz, RS Hess. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA

The purpose of this study was to determine whether aspart insulin is safe and effective for treatment of diabetic ketoacidosis (DKA) in dogs.

Twelve client-owned dogs with spontaneous DKA were enrolled into this prospective randomized clinical trial if they had blood glucose > 250mg/dl, venous pH between 7.0-7.35, and a blood beta-hydroxybutyrate concentration > 2.0mmol/L. Dogs were randomly assigned to receive an IV continuous rate infusion (CRI) of either aspart or regular insulin at an initial dose of 0.09 U/kg/hr, which was adjusted as previously described. Mean time intervals from the time the CRI of insulin was begun until resolution of marked hyperglycemia (blood glucose concentration > 250 mg/dl), acidosis (venous pH < 7.35), or ketosis (beta-hydroxybutyrate concentration > 2.0 mmol/L) were compared in dogs that received aspart or regular insulin. The number of hypoglycemic events (blood glucose < 60 mg/dl) that occurred in the two insulin treatment groups was also compared.

Mean time to resolution of hyperglycemia ($p = 0.29$), acidosis ($p = 0.26$), and ketosis ($p = 0.20$) were not significantly different between the two treatment groups. No hypoglycemic events were noted in either treatment group, and no other adverse effects were observed in association with IV aspart or regular insulin administration.

Should regular insulin become unavailable, aspart insulin is a safe and effective alternative for treatment of DKA in dogs.

## ABSTRACT EN-6

**DETERMINATION OF REFERENCE VALUES FOR CASUAL BLOOD GLUCOSE CONCENTRATION IN CLINICALLY-HEALTHY, AGED CATS MEASURED WITH A PORTABLE GLUCOSE METER FROM AN EAR OR PAW SAMPLE.** MK Reeve-Johnson[1], JS Rand[1], S Anderson[3], RD Marshall[2], D Vankan[1]. [1]Centre for Companion Animal Health, School of Veterinary Science, University of Queensland, Australia, [2]The Cat Clinic, Brisbane, Australia, [3]School of Biomedical Science, University of Queensland, Australia

Pre-diabetes is used to describe an intermediate stage between normal glucose homeostasis and type 2 diabetes. Pre-diabetes is estimated to be 4 times more prevalent than diabetes in the human population and pre-diabetic patients are at high risk of developing diabetes. Criteria for diagnosis of human pre-diabetes are either impaired fasting glucose (> 6.1 to < 7 mmol/L; > 110 to > 126 mg/dL after an 8 hour fast) or impaired glucose tolerance (7.8 ≤ 11.1 mmol/l; 140mg/dL two hours following an oral glucose load). A casual—or non-fasted—blood glucose concentration can be used as a screening test; scores > 11.1 mmol/l (200mg/dl) are considered abnormal. The incidence of pre-diabetes in cats is unknown, and there is a need to develop diagnostic criteria and screening tests for this in cats. Such criteria are especially important for aged cats because they are most at risk of diabetes. As cats are susceptible to stress hyperglycemia and fasting samples are often impractical to obtain, the diagnostic methodology should minimize stress and be based on causal blood glucose concentrations. Previous studies in cats have documented no statistical difference in blood glucose concentrations in capillary blood and venous blood.

The aim of this study was to determine reference values for casual blood glucose concentration in samples obtained in healthy cats ≥ 8 years old from a paw or ear sample (study method), and to compare these with values obtained from a jugular sample after a physical examination (traditional method). Clinically healthy cats (n = 74) aged ≥ 8 years old were recruited for the study; 8 cats were excluded due to concomitant medical problems or owner withdrawal, and 66 cats were enrolled. A casual blood sample was taken from the ear or paw at the beginning of the consultation. Blood glucose was measured immediately after sample collection using a portable glucose meter calibrated for feline blood (AlphaTRAK, Abbott Laboratories). A physical examination was performed before a further blood sample was taken from the jugular vein.

The inter- and intra-assay coefficients of variation for the glucose meter were 2% and 3.3% respectively. Mean blood glucose was 5.6 mmol/l (100.8 mg/dl; range 3.5-10.3mmol/l) for the study method and 6.4 mmol/l (115.2 mg/dl; range 4.2-10.7 mmol/l) for the traditional method. The upper limit of the 90% confidence interval for blood glucose for the study method was 9.7 mmol/L (174 mg/dl) for all breeds excluding Burmese, and 10.1 mmol/l (182mg/dl) for Burmese cats. Blood glucose concentrations were 0.7 mmol/L(13 mg/dl) lower (95% confidence interval 0.39 to 1.0 mmol/L; $p < 0.05$) when measured by the study method compared with the traditional method (72% of cats had a lower value). This study is the first to report the upper reference value for casual blood glucose concentration in aged, healthy cats, and demonstrates a simple method for screening for pre-diabetes in cats.

## ABSTRACT EN-7

**EARLY-ONSET HYPOADRENOCORTICISM IN A KINDRED OF POMERANIANS.** ET Mooney[1,2], TN Hammond[1], OM Mahony[2]. [1]Tufts Veterinary Emergency Treatment and Specialties, Walpole MA, [2]Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA

Hypoadrenocorticism (Addison's disease) is an uncommon endocrine disorder in dogs that typically affects middle-aged females. Familial hypoadrenocorticism has been demonstrated in several breeds including leonbergers, standard poodles, Nova Scotia duck tolling retrievers and Portuguese water dogs. The

purpose of this report is to describe a family of pomeranians with early-onset hypoadrenocorticism.

Three pomeranians, full siblings from two litters, were presented at 12 months, 17 months, and 26 months of age with clinical signs and laboratory findings consistent with hypoadrenocorticism including cardiovascular collapse and severe hyponatremia (125mmol/L, 118mmol/L and 115mmol/L). One dog was female and the others were male. ACTH stimulation testing confirmed hypoadrenocorticism in all dogs. Dogs were treated with intravenous fluid resuscitation and glucocorticoid and mineralocorticoid replacement and made a complete recovery.

Both parents and the other 4 siblings are currently healthy, and have never exhibited signs of hypoadrenocorticism.

This is the first report of an early-onset hypoadrenocorticism in pomeranians. An inherited basis is proposed to be the cause however further documentation will be required to confirm a breed predisposition.

## ABSTRACT EN-8

## CLINICAL FEATURES OF HYPOADRENOCORTICISM IN SOFT-COATED WHEATEN TERRIERS: 72 CASES (1979-2011).

RL Haviland, RL Toaff-Rosenstein, MP Reeves, MP Littman. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA

This retrospective study evaluated the clinical features of spontaneous hypoadrenocorticism in Soft-coated Wheaten Terriers (SCWT), a breed with increased risk. Medical records of 98 client-owned SCWT from North America diagnosed with hypoadrenocorticism were reviewed. Inclusion criteria were met in 72 cases (39 male, 33 female) including ACTH stimulation test results consistent with hypoadrenocorticism (pre-, post-cortisol $<2.0$ ug/dl), serum electrolyte values recorded at diagnosis, and absence of exogenous corticosteroid administration $>1$ month prior to diagnosis. Signalment, history, clinical signs, physical examination (PE) findings, clinicopathologic data, survival time, and concurrent disease were reviewed. Median age at diagnosis was 4.6 years (range 0.5- 11.4 years). Common clinical signs were lethargy (77%), anorexia (74%), vomiting (66%), and diarrhea (30%). The PE was unremarkable in 41% of cases; common abnormalities were dehydration (24%), depressed mentation (17%), thin body condition (15%), and bradycardia (4%). Electrolyte findings included Na/K ratio $\leq 30$ (96%), Na/K ratio $\leq 27$ (85%), hyperkalemia (77%), hyponatremia (63%), or no abnormalities (12%). The lymphocyte count was $\geq 2.0$ x 10^3 in 49 of 58 patients (84%). Median survival time following diagnosis was 3.2 years (range 0-8.6 years). Cause of death was largely unrelated to hypoadrenocorticism. The most common concurrent disease was protein-losing nephropathy (19%). Compared with prior studies concerning the general canine population with spontaneous hypoadrenocorticism, this study population showed no gender predisposition, less likelihood of serum electrolyte abnormalities at the time of diagnosis, and shorter median survival time.

## ABSTRACT EN-9

## THE EFFECT OF SURGERY ON THE PITUITARY-ADRENAL AXIS IN DOGS.

EJ Skovira, EN Behrend, L Palmer, LG Martin, RJ Kemppainen, HP Lee, Y Ueda. Auburn University, College of Veterinary Medicine, Auburn, AL

Pituitary-adrenal response to surgery has received limited study in dogs. Studies in humans indicate surgery activates the axis and may serve as a model for acute, moderate stress. In addition, it is important to know the level of axis activation upon post-operative entry into an intensive care unit in case testing of the axis is performed. Thus, our objective was to assess and compare the effect of surgery on serum cortisol, aldosterone, and ACTH concentrations in dogs.

Forty healthy dogs undergoing laparotomy for veterinary student surgical laboratories were used. Blood samples were obtained pre-operatively prior to administration of any medication and at the completion of the surgery with an average surgery time of 3 hours. Baseline samples were collected at least 4 hours prior to the start of surgery. Cortisol, aldosterone, and endogenous ACTH concentrations were measured using previously validated radioimmunoassays. Delta concentrations (pre-operative concentration subtracted from the post-operative) were calculated for each hormone. Data were analyzed using the paired t-test, Wilcoxon signed rank test, and Spearman correlation or linear regression. Significance was set at $p \leq 0.05$.

Cortisol, aldosterone, and ACTH concentrations increased significantly during surgery ($p \leq 0.001$ for all). However, while cortisol and aldosterone concentrations rose during surgery in all dogs, ACTH concentrations decreased in 26%. All dogs had a basal cortisol concentration within the reference range pre-operatively, but 63% had cortisol concentrations above the reference range post-operatively. A weak non-linear correlation was detected between pre-operative cortisol and ACTH concentrations; no correlation was evident post-operatively. A weak correlation between post-operative cortisol and aldosterone concentrations (linear regression, $p < 0.001$, $R^2 = 0.27$) and a moderate correlation between the delta cortisol and aldosterone values were detected (linear regression, $p < 0.001$, $R^2 = 0.34$). No relationship was detected between changes in either cortisol and aldosterone concentrations with ACTH.

In conclusion, laparotomy stimulates adrenocortical secretion of cortisol and aldosterone in dogs. In the majority of dogs, pituitary secretion of ACTH rises, but not in all. The lack of correlation between cortisol and ACTH concentrations post-operatively suggest the possibility that ACTH-independent mechanisms are responsible for the increase in post-operative cortisol values, or that an early increase in ACTH caused a sustained release of cortisol. The same mechanism may be at play, however, in causing increases in both cortisol and aldosterone secretion. Further studies are indicated to evaluate the effects of various anesthetic protocols and minimally invasive surgical techniques on the stress response.

## ABSTRACT EN-10

## LONG-TERM FOLLOW-UP OF RENAL FUNCTION IN DOGS WITH ACTH-DEPENDENT HYPERADRENOCORTICISM.

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Systemic hypertension and proteinuria are frequent complications in dogs with Cushing's syndrome and do not always resolve after treatment of hypercortisolism. Therefore, these patients may be at risk for renal dysfunction.

Objectives were to assess renal function in dogs with ACTH-dependent hyperadrenocorticism (ADHAC) before and after treatment. A total of 19 dogs with ADHAC were included. Renal function was assessed before, and at 1, 3, 6, and 12 months after treatment. Twelve dogs were treated with trilostane and 7 dogs by transsphenoidal hypophysectomy. Routine renal markers were measured and urinary albumin (uALB), immunoglobulin G (uIgG), and retinol-binding protein (uRBP) were assessed by ELISA. Urinary N-acetyl-β-D-glucosaminidase (uNAG) was determined colorimetrically. All urinary markers were indexed to urinary creatinine concentration (c). Plasma clearance of creatinine ($Cl_{creat}$), exo-iohexol ($Cl_{exo}$), and endo-iohexol ($Cl_{endo}$) were used tomeasure glomerular filtration rate (GFR). Data were analyzed using a general linear model.

Serum creatinine and urea concentration increased post treatment, but remained within reference ranges. Plasma $Cl_{creat}$ and $Cl_{endo}$ were significantly lower post treatment, whereas $Cl_{exo}$ was not different. Urinary protein-to-creatinine ratio, uALB/c, uIgG/c, and uRBP/c were decreased post-treatment, but at 12 months 5/13 dogs remained proteinuric. Urinary NAG/c did not change significantly.

The decrease in GFR and persistent proteinuria post-treatment underline that canine ADHAC is associated with renal glomerular and tubular changes which do not always reverse after therapy. (ESVE Award Winner).

**ABSTRACT EN-11**
**EVALUATION OF A LONG-ACTING SOMATOSTATIN RECEPTOR LIGAND FOR THE TREATMENT OF FELINE ACROMEGALY.** J Timian, KF Lunn. Department of Clinical Sciences, Colorado State University, Fort Collins, CO

This study was designed to evaluate whether long-acting octreotide (Sandostatin LAR®) is an effective medical therapy for cats with acromegaly.

Seven cats were diagnosed with acromegaly, based on physical changes, the presence of insulin-resistant diabetes mellitus (diabetes poorly regulated on $\geq 5$ units of insulin BID), elevated insulin-like growth factor 1 (IGF-1) levels, and no evidence for other causes of insulin resistance. Cats received monthly long-acting octreotide IM at an escalating dose of 2 mg for 3 months, 3 mg for 1 month, and 4 mg for 2 months. Body weight, insulin dose, biochemistry profile, IGF-1 level, and fructosamine were obtained prior to each injection, and at one month after the last injection of long-acting octreotide.

Four cats received medication for 6 months; 3 cats received medication for 3 months (1 cat euthanized due to progressive disease; 1 cat withdrawn by the owner; 1 cat still enrolled). Mean serum IGF-1 levels, before and after the course of therapy, were 334 nmol/l and 339 nmol/l respectively. Mean serum fructosamine values before and after therapy were 426 umol/l and 435 umol/l respectively. Mean insulin doses before and after therapy were 1.4 units/kg and 1.1 units/kg respectively. A paired *t*-test showed no significant differences between any of these parameters before and after therapy ($p = 0.94$; $p = 0.77$; $p = 0.14$, respectively). One cat became non-insulin-dependent, coinciding with marked obtundation; necropsy revealed hemorrhage within and around a pituitary tumor. Six cats remained significantly insulin resistant. Long-acting octreotide does not appear to be an effective treatment for feline acromegaly.

**ABSTRACT EN-12**
**BASAL ALDOSTERONE CONCENTRATIONS AND RESPONSE TO ACTH STIMULATION IN HEALTHY AND CRITICALLY ILL DOGS.** LG Martin, EN Behrend, RJ Kemppainen, HP Lee. Auburn University, College of Veterinary Medicine, Auburn, AL

Critical illness-related corticosteroid insufficiency (CIRCI) has been recognized in veterinary patients. In humans diagnosed with CIRCI, it is generally accepted that aldosterone insufficiency is not a part of the syndrome. The same is suspected to occur in dogs with CIRCI, but has not been documented. Furthermore, aldosterone concentrations and its response to ACTH are infrequently measured in ill dogs despite the life-threatening effects of aldosterone insufficiency. The purpose of this study was to determine basal aldosterone concentrations and their response to ACTH in clinically healthy and critically ill dogs, and to determine the relationship between cortisol and aldosterone insufficiency in dogs with CIRCI.

Basal and post-ACTH aldosterone concentrations were determined in 10 healthy and 32 critically ill dogs diagnosed with sepsis, trauma, or GDV. (Cortisol data reported in Martin LG et al, JVIM, 2010.) Each dog was given cosyntropin (5 µg/kg IV) and blood samples for assay of aldosterone and cortisol concentrations were obtained before and 60 minutes after administration. Serum sodium and potassium concentrations as well as delta aldosterone and cortisol concentrations (the difference between stimulated and basal concentrations) were also determined. Data were analyzed using Kruskal-Wallis one-way ANOVA, Mann-Whitney rank sum test, Wilcoxon signed rank test, and Spearman rank order correlation. Significance was set at $p \leq 0.05$.

Undetectable basal or post-ACTH aldosterone concentrations were found in 18/32 (56%) critically ill dogs. Ten of the critically ill dogs met biochemical criteria for the diagnosis of CIRCI based on their cortisol concentrations. Only 2/10 had either a low basal or post-ACTH aldosterone concentration. None of the dogs diagnosed with CIRCI or aldosterone deficiency had hyponatremia or hyperkalemia. Median basal aldosterone concentrations in dogs with GDV were significantly lower when compared to healthy and septic dogs. In septic and trauma dogs, no difference was detected between median basal and post-ACTH aldosterone concentrations. Median delta aldosterone concentrations in the trauma dogs were lower than those of healthy dogs. No relationship was detected between basal, post-ACTH, and delta aldosterone and cortisol concentrations or between basal aldosterone and electrolyte concentrations.

In conclusion, low aldosterone concentrations were common in this population of critically ill dogs. The majority of dogs with CIRCI did not have decreased aldosterone concentrations; thus, canine CIRCI may be similar to the human syndrome. The etiologies for low aldosterone concentrations in this population of critically ill dogs are unknown at this time, but do not appear to be due to abnormalities in sodium or potassium. Future studies are needed to determine the effects of critical illness on adrenocortical aldosterone secretion and the significance of the aldosterone deficiency.

**ABSTRACT EN-13**
**EVALUATION OF ONCE-DAILY ADMINISTRATION OF INSULIN GLARGINE IN DOGS WITH NATURALLY OCCURING DIABETES MELLITUS.** AY Kim, K Cho, S Noh, J Paek, M Lee, D Chang, MP Yang, JH Kang. Chungbuk National University College of Veterinary Medicine, Cheongju, Chungbuk, Republic of Korea

The objective of this study was to evaluate the efficacy of once-daily administration of insulin glargine in dogs with diabetes mellitus (DM). Sixteen client-owned dogs with DM were enrolled, all of which had been previously treated with neutral protamine Hagedorn (NPH) insulin for at least 4 weeks following diagnosis of DM. Nine of these dogs (Group A) had DM that was well-regulated by twice-daily administration of NPH insulin. In the other seven dogs (Group B) NPH showed short-duration of action at the lowest dose. Dogs received insulin glargine (Lantus®) every 24 hours and were fed the recommended prescription diet. Evaluations were performed prior to treatment with insulin glargine and 1, 2, 4, 6, and 10 weeks after the administration of insulin glargine. Evaluations included clinical signs, blood glucose curves and serum fructosamine concentrations. Adjustments in dosage of insulin glargine were made as needed to control glycemia.

The 24-hour mean blood glucose (MBG) and serum fructosamine concentrations in Group A were significantly elevated 2 weeks and 4 weeks after initiating the treatment, respectively, as compared to levels prior to the administration of insulin glargine. In Group B, by 4 weeks after initiation of treatment there were no significant differences in MBG and serum fructosamine concentrations as compared to prior to treatment, and no recurrence of polyuria/polydipsia. Maintenance of successful glycemic control was attained in only seven of all dogs (one dog in Group A and six dogs in Group B). The once-daily administration of insulin glargine is not generally effective in diabetic dogs but may possibly be used as an alternative treatment in diabetic dogs in which insulin has only a short-duration of action at the lowest dose.

**ABSTRACT EN-14**
**CHROMATOGRAPHIC ANALYSIS OF THE LIPID FRACTIONS IN OBESE CLIENT-OWNED DOGS.** MM Jericó[1], FC Chiquito[1], F Fusco[2], S Catanozi[2], VS Nunes[2], ER Nakandakare[2]. [1]Anhembi Morumbi University and Cruzeiro do Sul University, SP, Brazil, [2]Lipids Laboratory LIM 10 of Faculty of Medical Sciences of the University of Sao Paulo, SP, Brazil

Obesity, a metabolic disease of multifactorial origin, impairs the health and longevity of individuals. Dyslipidemia, a common condition in obese animals, is usually associated to a variety of

clinical problems, which include gastrointestinal alterations, hepatic lipidosis, atherosclerosis and metabolic syndrome. The aim of the study was to characterize the lipid profile and lipoprotein fractions in obese dogs (n = 20) and compare them with control dogs (n = 10), selected from a veterinary hospital environment. Lipoproteins (LP) were isolated by fast protein liquid chromatography (FPLC) system and plasma total cholesterol (TC) and triglycerides (TG) concentrations were measured by enzymatic methods. In control group, TC was 193±44 mg/dL (mean±SD) (percentage distribution among LP fractions: VLDL-C 2.34%, LDL-C 15.79%, HDL-C 81.86%); Plasma TG concentration was 50±15 mg/dL (LP fraction percentage distribution: VLDL-TG 39.17%, LDL-TG 35.9%, HDL-TG 24.94%). In obese dogs, plasma TC was 227±73 mg/dL (VLDL-C 3,325%, LDL-C 21.64%, HDL-C 75.03%) whereas the TG values were 87.9±52 mg/dL (VLDL-TG 61.98%, LDL-TG 24.71%, HDL-TG 13.36%). When compared to the normal dogs, the animals with obesity presented a significant increase ($p < 0.01$) in plasmaTG and VLDL-TG levels as well as a lower HDL-TG concentration. There were not significant alterations in cholesterol fractions or TC between groups although they presented proportionally higher levels of TC, and LDL-C as well as lower HDL-C. It was concluded that obese dogs present a significant difference when compared to normal dogs in relation to triglyceride metabolism and their VLDL and HDL fractions, which has been previously shown in experimental model of obese canine insulin resistance. These findings suggest a higher risk of metabolic complications in obese client-owned dogs, like diabetes, hepatobiliary diseases and of blood flow disturbances, like atherosclerosis.

**Key words:** dogs, obesity, lipoproteins, cholesterol, triglycerides.

**ABSTRACT EN-15**

**CHROMATOGRAPHIC ANALYSIS OF THE LIPID FRACTIONS IN OBESE CLIENT-OWNED CATS.** MM Jericó[1], S Zbóril[1], ER Nakandakare[2], VS Nunes[2], S Catanozi[2], CA Geraldo Jr[3], ANL Wludarski[4], R Soila[5], P Furtado[5]. [1]Anhembi Morumbi University, Cruzeiro do Sul University, SP, Brazil, [2]Lipids Laboratory LIM 10 of Faculty of Medical Sciences of the University of Sao Paulo, SP, Brazil; , [3]Vetmaster Clinic and FMVZ-USP University, SP, Brazil;, [4]Petcenter Clinic, SP, Brazil; , [5]PROVET Laboratory, SP, Brazil

Obesity, a metabolic disease of multifactorial origin, impairs the health and longevity of individuals. Dyslipidemia, a common condition in obese animals, is usually associated to a variety of clinical problems, which include gastrointestinal alterations, hepatic lipidosis, atherosclerosis and metabolic syndrome. The aim of the study was to characterize the lipid profile and lipoprotein fractions in obese cats (n = 9) and compare them with control cats (n = 10), both selected from a veterinary hospital environment. Lipoproteins (LP) were isolated by fast protein liquid chromatography (FPLC) system and plasma total cholesterol (TC) and triglycerides (TG) concentrations were measured by enzymatic methods. In control group, TC was 132±59 mg/dL (mean±SD) (LP fraction percentage distribution: VLDL-C 4.9%, LDL-C 28.3%, HDL-C 66.9%); Plasma TG concentration was 57±25 mg/dL (LP fraction percentage distribution: VLDL-TG 32.8%, LDL-TG 35.5%, HDL-TG 31.2%). In obese cats, plasma TC was 167±71 mg/dL (VLDL-C 5,3%, LDL-C 31.3%, HDL-C 63.3%) whereas plasma TG concentration was 93.5±43 mg/dL (VLDL-TG 43.2%, LDL-TG 30.3%, HDL-TG 26.5%). Total TG and VLDL-TG were higher in obese cats as compared to control group (t Student test; $p < 0.05$). On the other hand, TC, VLDL-C, HDL-C, LDL-C, HDL-TG, LDL-TG values were not different between animal groups, although they presented proportionally higher levels of TC, LDL-C as well as higher HDL-C. Similar results have been previously shown in experimental model of obese feline insulin resistance These findings suggest a higher risk of metabolic complications in obese client-owned cats, like diabetes and hepatobiliary diseases.

**Key words:** cats, obesity, lipoproteins, cholesterol, triglycerides.

# FOOD ANIMAL

**ABSTRACT FA-1**

**SKILLS REQUIRED OF ENTRY LEVEL VETERINARIANS: A SURVEY OF DAIRY PRACTITIONERS.** C Luby, K McIntyre, M Jelinski. Western College of Veterinary Medicine, Saskatoon, SK

To provide the optimal training experience for veterinarians, it is important to determine the skills expected at graduation. The purpose of this study was to determine skills required of entry-level veterinarians for dairy practice in Western Canada.

We performed a survey of Western Canadian veterinarians involved in dairy practice. The survey included questions regarding respondent's clinical activity, demographics, skills performed in practice, open ended questions and space for comments. All work was approved by the University of Saskatchewan Behavioural Research Ethics Board.

Response rate for the surveys was 39.3% (281/714 mailed out). Of these, five were excluded due to inconsistencies in answers. Of the respondents, 43.8% were involved in dairy practice with a median 15% (0.5 – 100%) of practice time involving dairies. Respondents were classified as either mixed practitioners (< 25% time in dairy practice) or dairy practitioners (> 75% time in dairy practice). For both groups, individual animal medicine and surgery skills were performed more commonly than production medicine skills. The most important skills identified were pregnancy diagnosis by rectal palpation or ultrasound, correction of displaced abomasum, physical examination and treatment of common disorders.

These results demonstrate the continued importance of individual animal skills in dairy practice. Training programs for veterinarians should provide high quality training in individual animal medicine, showing that ACVIM diplomates can provide an important role in preparing students for dairy medicine.

**ABSTRACT FA-2**

**METAGENOMIC EVALUATION OF THE INTESTINAL MICROBIOME IN VEAL CALVES AND THE INFLUENCE OF ORAL TETRACYCLINE.** MC Costa, HR Staempfli, LG Arroyo, FLF Feitosa, JS Weese. University of Guelph, Guelph, ON. Canada, Universidade Estadual Paulista, Araçatuba. Brazil

The intestinal bacterial population (the microbiome) is a complex polymicrobial community that has an important role in many aspects of health and disease. While there has only been superficial study of the intestinal microbiome in most species, recent advances in molecular technologies have allowed for comprehensive evaluation of the microbiome, including unculturable bacteria not identified before, and detailed investigation of changes in the microbiome in response to various factors, such as antimicrobials. The objective of this study was to characterize the intestinal microbiome of veal calves by high throughput sequencing technology, and to investigate the impact of oral tetracycline on the intestinal microbiome.

Fecal samples were collected from 22 healthy Holstein male calves (2-7 days of age) within 24h after arrival on a commercial veal farm. 12 calves were then treated with oxytetracycline (6.6 mg/kg PO for 5d) and 10 control calves did not receive any antimicrobials. A 2nd fecal sample was collected 2 days after treatment was finished. DNA was extracted and PCR of the 16S rRNA gene was performed. Amplicons were sequenced by high throughput sequencing technology with the use of a 454 sequencer. The MOTHUR package of algorithms was used to clean data and align sequences with the SILVA 16S rRNA reference database, with taxonomic classifications obtained from the Ribosomal Database Project. Sequences were assigned into operational taxonomic units (OTUs) using the average neighbor algorithm.

From 133,943 sequences obtained, 110,274 high quality sequences remained after data cleaning. For OTU classification, a minimum of 2,324 sequences per calf was used, which

excluded 3 calves from the analysis. Bacteroidetes was the main Phylum among control and treated calves before use of oxytetracycline (65.7 and 46.9%, respectively) with Firmicutes being the second most prevalent (33 and 29%, respectively). There was a profound decrease in abundance of Bacteroidetes in both control ($P = 0.003$) and treated ($P = 0.014$) calves at the 2nd sampling time, with post-treatment abundances of 0.6 and 1.6%) and a concurrent increase in Firmicutes in control ($P = 0.012$) and treated ($P = 0.035$) calves to 60.8 and 92.4%, respectively. Different communities were found between 1st and 2nd sampling in both control ($P < 0.013$) and treated calves ($P < 0.001$), as well as between the two groups at the 2nd sampling ($P < 0.001$).

While there was difference between treatment and control groups, the fecal microbiome changed dramatically in the 7 day period after farm arrival irrespective of tetracycline exposure. Multiple factors may be involved in fecal microbiome alterations and these must be considered when assessing the impacts of antimicrobial therapy or other potential modifying factors.

## ABSTRACT FA-3

**A PROSPECTIVE CONTROLLED STUDY ON PREVALENCE OF *CLOSTRIDIUM DIFFICILE* IN VEAL CALVES RECEIVING AND NOT RECEIVING ORAL OXTETRACYCLINE.** MC Costa, H Staempfli, LG Arroyo, FLF Feitosa, JS Weese. University of Guelph, Guelph, ON. Canada, Universidade Estadual Paulista, Araçatuba. Brazil

*Clostridium difficile* is the major cause of antibiotic associated diarrhea in humans. While the role of this bacterium in disease in calves is still unclear, public health concerns have been expressed because of its presence in food animals and food. Epidemiologic studies have shown an increase on the prevalence of *C. difficile* in veal calves after arrival on the farm, which coincides with antimicrobial treatment. Whether this increase is physiological or due to treatment remains uncertain. The objective of this prospective trial was to compare the prevalence of *C. difficile* in veal calves treated with oral oxytetracycline compared to age matched controls.

Twenty-two bull calves (2-7 days) arriving on a veal farm were enrolled. They were clinically normal and housed in individual pens. Twelve received oral oxytetracycline (6.6mg/kg PO for 5d) starting at arrival, and ten calves were left untreated. Feces were collected by rectal digital stimulation at arrival, 3 days into treatment and 2, 10 and 27 days after treatment. Selective culture for *C. difficile* was performed. Fisher's exact test was used to determine the relationship between treatment with oral oxytetracycline and the shedding of *C. difficile*.

The prevalence of *C. difficile* in both groups at the different sampling times is presented on Table 1. No significant statistical differences were found between groups at any sampling times. The prevalences found at the first two samplings were consistent with previous reports in the literature. Interestingly, an increase occurred at the fifth sampling, which coincided with the moving of the calves to collective pens (groups of 10) in another room and therefore submitted to stress.

Administration of oral oxytetracycline seems to have no impact on the prevalence of *C. difficile* in calves and the changes in prevalence that have been noted may be due to other factors, such as environmental exposure, external stressors and other factors that may affect the intestinal microflora.

**Table 1.** Prevalence of *C. difficile* in veal calves and *P* values before and after administration of oral oxytetracycline.

| | 1st | 2nd | 3rd | 4th | 5th |
|---|---|---|---|---|---|
| Control (%) | 5/10 | 5/10 | 8/10 | 1/10 | 4/10 |
| Treatment (%) | 1/12 | 6/12 | 7/12 | 3/12 | 7/12 |
| *P* value | 0.056 | 1.000 | 0.381 | 0.594 | 0.670 |

## ABSTRACT FA-4

**INCREASED STRONG ION GAP IN DAIRY CALVES WITH DIARRHEA IS LIKELY DUE TO D-LACTATE.** HR Staempfli[1], PD Constable[2]. [1]Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada hstaempf@uoguelph.ca, [2]Purdue University, West Lafayette, Indiana

Physicochemistry emphasizes the importance of strong electrolytes (Na, K, Cl, L-lactate, D-lactate), $pCO_2$, and plasma protein concentrations in determining plasma pH. We recently determined that the physicochemical approach provided a clinically useful method to predict the dependent variables (pH, $HCO_3$) in critically ill horses by using a Radiometer 800 Flex blood gas analyzer to measure the plasma concentration of strong electrolytes and refractometry to measure total protein concentration (TP) (*J Vet Intern Med* 25 2011 p674). The situation in calves with diarrhea might differ from that in horses in that calves frequently develop strong ion (metabolic) acidosis due to elevation of unmeasured strong anions such as D-lactate..

Eighty-three venous blood gas analysis samples obtained anaerobically from 20 calves aged 2 days to one month presented to the Veterinary Teaching Hospital for dehydration and acid base abnormalities associated with diarrhea were analyzed. Na-heparinized blood samples were analyzed for pH ($pH_m$), $pCO_2$ and concentrations of Na, K, Cl, and L-lactate using a Radiometer 800 Flex blood gas analyzer; Strong Ion Difference (SID4 = Na + K-Cl- Lac) was calculated and TP was estimated using refractometry. Plasma pH values ($pH_c$) were calculated using Constable's 6 factor simplified equation ((J. Appl. Physiol., 1997. *83*. 297-311). Anion gap (AG) and Strong ion gap (SIG) were defined as: $AG = (Na + K)-(Cl + HCO_3^-)$; $SIG = [(0.343xTP)/(1 + 10^{\{7.10\text{-}pH\}})]$-AG. Linear regression analysis was used to compare $pH_c$ to measured $pH_m$, as well as AG and SIG to blood L-lactate concentrations.

Measured pH ranged from 6.66 to 7.59 (7.27±0.16, mean±SD); measured SID4 ranged from 22.1 to 56.3 mEq/L (36.6±6.1 mEq/L); TP concentration ranged from 32-98 (53.5±13.3 g/L). $pH_c$ was poorly associated with $pH_m$ ($r = 0.10$; $pH_c = 0.31 + 5.09$). This result was probably due to the presence of unmeasured strong anions not included in the SID4 estimate, with the most likely unmeasured strong anion being D-lactate. Anion gap (in mEq/L) was significantly and positively correlated with L-lactate concentration (mmol/L); $AG = 0.93x$ [lactate] + 8.3 ($R^2 = 0.39$). SIG (mEq/L) was significantly and negatively correlated with L-lactate concentration $SIG = -1.10x$ [lactate]-4.4 ($R^2 = 0.25$). The intercept estimate of -4.4 mEq/L for the strong ion gap regression equation indicated that neonatal calves with diarrhea had a mean unmeasured strong anion concentration of 4.4 mEq/L.

Our findings demonstrate that a clinically useful application of strong ion difference theory is calculation of the SIG to quantify the unmeasured strong anion concentration. It would be interesting to prospectively determine that the unmeasured strong anion was D-lactate. We also conclude that an accurate estimate for strong ion difference is required if plasma pH is to be calculated from plasma constituents.

## ABSTRACT FA-5

**ANTIMICROBIAL RESISTANCE TRENDS AMONG *SALMONELLA* ISOLATES OBTAINED FROM DAIRY CATTLE IN THE NORTHEASTERN USA, 2004-2010.** KJ Cummings[1,2], GA Perkins[2], SM Khatibzadeh[2], JD Siler[2], LD Warnick[2], C Altier[2]. [1]Texas A&M University, College Station, TX, [2]Cornell University, Ithaca, NY

The objectives of this study were to describe the antimicrobial resistance status of *Salmonella* isolates recently obtained from dairy cattle in New York and other northeastern states and to identify trends in resistance to various antimicrobial agents over time.

Data were collected retrospectively for all bovine *Salmonella* isolates that were obtained from samples submitted to the Cor-

nell University Animal Health Diagnostic Center between January 1, 2004 and December 31, 2010 and that were subsequently tested for antimicrobial susceptibility. Standard bacteriologic culture methods were used to isolate *Salmonella* from samples, and antimicrobial susceptibility of isolates was determined by use of the microbroth dilution method. Temporal trends in the prevalence of resistant *Salmonella* were investigated for each antimicrobial agent using the Cochran-Armitage trend test.

Antimicrobial susceptibility testing was performed on 4,589 bovine *Salmonella* isolates during the study period. Resistance to individual antimicrobial agents ranged from 0% (amikacin) to 74.9% (sulfadimethoxine) of all isolates tested. The Cochran-Armitage test showed evidence of an overall significantly decreasing trend in prevalence of resistance to most antimicrobial agents (AUG-amoxicillin/clavulanic acid, AMP-ampicillin, FOX-cefoxitin, TIO-ceftiofur, AXO-ceftriaxone, CHL-chloramphenicol, FFN-florfenicol, CTET-chlortetracycline, OXY-oxytetracycline, TET-tetracycline, KAN-kanamycin, NEO-neomycin, SPE-spectinomycin, STR-streptomycin, and FIS-sulfisoxazole). Among the 1,677 isolates that were tested using the full National Antimicrobial Resistance Monitoring System (NARMS) panel, the most common resistance patterns were pan-susceptible (53.0%), AUG-AMP-FOX-TIO-AXO-CHL-KAN-STR-FIS-TET (10.1%), AMP-KAN-STR-FIS-TET (8.1%), and AUG-AMP-FOX-TIO-AXO-CHL-STR-FIS-TET (6.9%).

These results do not support the notion that there is an ongoing increase in the emergence and dissemination of resistant *Salmonella* isolates among dairy cattle.

**ABSTRACT FA-6**

**EVALUATION OF ORALLY SUPPLEMENTED D, L-METHIONINE AS A URINE ACIDIFIER FOR SMALL RUMINANTS.** G Grissett, S Fleming, K Neizman. Mississippi State University College of Veterinary Medicine, Mississippi State, MS

Urolithiasis is the most economically significant urinary disease of ruminants, specifically castrated male small ruminants. Due to treatment expense and poor prognosis for full recovery, focus is generally placed on prevention. Most prevention strategies attempt to create an environment inhibiting urolith formation such as an acidic urine pH. Methionine, a limiting amino acid for most livestock species, is well-documented in castrated male felids as a urine acidifier. This study investigated the ability of D, L-Methionine (Met) to acidify urine in small ruminants without significant clinically apparent side effects. The first objective was to determine if oral administration of Met would acidify urine and lower solute concentration. The second objective was to determine if castrated male goats (wethers) voluntarily would consume a sufficient quantity of Met added to grain to acidify urine and lower solute concentration. Five caprine wethers (4 Nubian, 1 Oberhasil) were administered Met (Ammonil®) orally at a 200mg/kg dose daily. One urine sample was collected pre-treatment and fourteen treatment samples were collected via free catch. The urine samples were analyzed for urine pH, specific gravity, and osmolality using an electric pH meter, refractometer, and osmometer respectively. Wethers were given a one week wash-out period between trials. Pre-treatment urine samples were obtained before beginning the second trial. The same dose of Met was added to the grain ration of each wether and urine samples were collected for fourteen treatment days and analyzed as previously described. Orally administered Met caused a mean drop in pH across individuals that ranged from 0.66-1.82. Within Trial 1 there were variations in individual urinary pH both up and down. At the end of Trial 1the urine pH change was 1.7 from pre-treatment mean. Trial 2 with voluntary intake of Met resulted in a significant continuous drop in urine pH with mean being 7.8 on trial day 1 and 5.7 on trial day 14. The mean urine pH change in trial 2 ranged from 0.51-1.59. Three wethers reliably ate the dose of methionine while two increased their intake during Trial 2 to eating the full dose by completion of the trial. No other clinically apparent side effects were noted. Fluctuations in urine pH may suggest a renal compensatory mechanism to the Met. Neither orally administered or feed added Met reliably decreased urine solute concentration based on specific gravity and osmolality. Based on these results, Met has potential promise as a more palatable alternative for urine acidification in small ruminants that is worthy of further investigation.

**ABSTRACT FA-7**

**THE EFFECT OF AMMONIUM CHLORIDE TREATMENT AS A LONG TERM PREVENTATIVE APPROACH FOR UROLITHIASIS IN GOATS AND A COMPARISON OF CONTINUOUS AND PULSE DOSING REGIMES.** P Sprake, AJ Roussel, R Stewart, WT Bissett. Texas A&M University, College Station, Texas

Long-term oral treatment with ammonium chloride in goats to acidify urine as a mechanism to prevent phosphatic urolithiasis has been observed to lose clinical effectiveness. Clinically, pulse dosing has been utilized in order to prevent the failure to achieve acidification of urine as seen with continuous daily dosing.

We hypothesized that continuous daily dosing of ammonium chloride would lose clinical effectiveness, pH < 6.5, within 7 days, and that pulse therapy (3 days of treatment followed by 4 days rest) would result in repeated attainment of target pH during the 3-day treatment periods.

10 wether goats fed a commercially available concentrate feed containing 0.1% ammonium chloride and supplemented with alfalfa hay, were treated with ammonium chloride as continuous daily dosing and pulse regimes in a cross over study design. The dose of ammonium chloride was titrated for each goat, starting at 200 mg/kg q 12 hours, and increasing to 250 mg/kg and/or 300 mg/kg q12 hours in order to determine a dose that decreased the pH to below target within 3 days. Urine was collected twice daily at each treatment time by use of a cup collection system and pH was determined using a 3-pad indicator strip and pH meter. Once an effective dose had been determined for each goat, continuous twice daily dosing was administered for 10 days, and pulse dosing was continued for 3 treatment cycles. Basic descriptive statistics were performed for this data.

The median urine pH for all goats during the pre-treatment period was 8.3. Twenty four hours after starting continuous twice daily treatment, 88% (8/9) of the goats had urine pH below the target, achieving a median pH nadir of 5.49 (range 4.32-7.61). Following 4 days of continuous dosing, only 50% (4/8) goats had urine < 6.5. Mean urine pH remained above the target pH for subsequent treatment days, until 8.5 days after starting treatment when median urine pH returned to below the target pH. 60 hours after ammonium chloride treatment was discontinued, all goats had urine pH of > 6.5. During the pulse treatment regime, target median urine pH was achieved in cycles 1 and 3, 30 hours after initiating treatment each. 4/10 goats achieved urine pH < 6.5 for all treatment cycles. All received pulse dose as the first treatment regime suggesting that treatment order influenced urine pH.

These results showed that ammonium chloride is an effective method of acidifying urine in goats if the dose is titrated for each individual. The data demonstrated re-alkalinization does occur in goats receiving continuous daily dosing of ammonium chloride, as has been suggested by previous literature. In this study, re-alkalinization occurred at day 4 of ammonium chloride treatment. For some goats pulse dosing repeatedly achieved urine pH to below the target, and therefore pulse dosing regimens may be effective in prolonging the treatment effect of ammonium chloride in the prevention of phosphatic urolithiasis.

**ABSTRACT FA-8**
**ECHOCARDIOGRAPHIC MEASUREMENT OF VALVULAR THICKNESS IN HEALTHY COWS, COWS WITH BACTERIAL ENDOCARDITIS AND COWS WITH CARDIORESPIRATORY DISEASES.** S Buczinski[1], M Tolouei[2], A Rezakhani[3], T Mohamed[4]. [1]Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada, [2]Department of Clinical Studies, School of Veterinary Medicine, University of Tabriz, Iran, [3]Department of Clinical Studies, School of Veterinary Medicine, Shiraz University, Shiraz, Iran, [4]Faculty of Veterinary Medicine, Zagazig University, Egypt

Ante-mortem diagnosis of bacterial endocarditis (BE) is based on different signs including the echocardiographic observation of valvular thickening. This finding is a subjective criterion especially for operator not used to echocardiography. Our objective was to determine the valvular thickness in healthy cows, cows with a diagnosis of BE and cows with various cardiorespiratory diseases.

Forty healthy Holstein adult cows (CONTROL), 12 adult cows with BE (BE) and 10 cows with other cardiorespiratory disorders (NONBE).The valvular thicknesses were assessed in 4 different locations for all the cardiac valves and the maximal value was used for further analysis.Results: The mean (±SD) maximal thicknesses of the tricuspid, mitral, aortic and pulmonary valves in CONTROL were 0.69±0.10 cm, 0.85±0.21 cm, 0.72±0.17 cm, and 0,58±0.12 cm, respectively. The maximal valvular thicknesses of the tricuspid and pulmonary valves were less than 1 cm and for mitral and aortic valves were less than 1.28 cm. The mean (±SD) maximal thicknesses of the affected valves in BE group were 4.50±1.25 cm; 4.04±1.32 cm, 5.16 cm, 2.80 ±1.24 cm for the tricuspid (n = 6), mitral (n = 3), aortic (n = 1) and pulmonary (n = 2) valves, respectively. This was different from CONTROL group as well as the normal valves in BE group for tricuspid valve (P = 0.0006) but not for the mitral (P = 0.055) or pulmonary valve (P = 0.25). The maximal valvular thickness of NONBE as well as non affected valves in BE group were not different from the CONTROL group. The maximal valvular thickness in healthy dairy cows is generally less than 1cm when assessed by right transthoracic echocardiography using low frequency phased array probe. Thicker valvular measurement should therefore raise the possibility of valvular endocarditis.

**ABSTRACT FA-10**
**UTERINE NEOPLASIA IN SMALL RUMINANTS: RETROSPECTIVE STUDY (1991-2011).** M Heller. University of Missouri College of Veterinary Medicine, Columbia, MO

Literature concerning uterine neoplasia in small ruminants is limited. The goal of this retrospective study is to add to our understanding and aid in the diagnosis of uterine neoplasia in sheep and goats by identifying common presentations, physical exam findings, and diagnostic results. Medical records of sheep and goats diagnosed with uterine neoplasia from 1991 to 2011 were reviewed. A total of 19 cases were reviewed for presenting complaints, history, physical exam findings, clinical pathology findings, diagnostic imaging results and histopathologic diagnosis. Cases lacking a definitive diagnosis were excluded. Age at presentation ranged from 4-14 years old with a mean of 9.16 years and a median of 10 years. All cases except one were goats, with the most common breed being Pygmy goats (74%). The most common presenting complaints were anorexia (63%) and vaginal discharge (70%), followed by straining (47%). On physical examination 46% of cases had palpable masses in the caudal ventral abdomen with 2 cases also being palpable in the paralumbar fossa area as well. Concurrent udder enlargement or inappropriate lactation was observed in 56% of cases. No distinct trends were noted upon analysis of CBC or serum biochemistry results, however for the subset of patients who had urinalysis performed 8 out of 9 had proteinuria and/or hemoproteinuria ranging from trace to 3+. Diagnostic imaging results were also analyzed, uterine abnormalities were visualized in 14 out of 15 cases undergoing abdominal ultrasonagraphic exam. Four of the 7 cases for which abdominal radiographs were taken had evidence of caudal abdominal mass. The most common histopathologic diagnosis was uterine adenocarcinoma (56%), other tumor types diagnosed were leiomyoma, leiomyosarcoma and leiomyofibroma. Interestingly, several of the adenocarcinoma cases had concurrent cystic endometrial hyperplasia as well as two cases who had previously undergone ovariohysterectomy for chronic cystic endometrial hyperplasia and were both diagnosed with adenocarcinoma of their uterine stumps. Thus endometrial hyperplasia may represent a pre-cancerous condition in goats. Uterine neoplasia should be a differential diagnosis for goats presenting with straining and vulvar discharge, particularly those with a history of udder enlargement not associated with pregnancy. Abdominal ultrasonagraphy is a useful diagnostic tool for evaluating reproductive tract of small ruminants.

**ABSTRACT FA-11**
**CAMELID HEAT STRESS AS A SYNDROME: 15 CASES (2003-2011).** PL Norton[1], JR Gold[2], KL Schulz[1], BF Porter[1]. [1]Texas A&M College of Veterinary Medicine, College Station, TX., [2]Dubai Equine Hospital, Dubai, UAE.

Heat stress is a well recognized condition in the southern United States bourgeoning camelid population. Camelids' limited thermal cooling window can be compromised by dense fiber when unshorn during periods of elevated environmental temperatures combined with high humidity. There is currently a paucity of peer reviewed literature available regarding clinical outcomes of camelids diagnosed with heat stress.

Medical records of camelids admitted to TAMU VMTH between 2003 and 2011 were reviewed. Criteria for inclusion included medical diagnosis, laboratory values and body temperature. Subject details, clinicopathologic findings, survival to time of discharge, treatments, and non-survivor necropsy findings were recorded. A heat stress index was calculated based on the recorded ambient temperature and humidity.

Fifteen camelids fit the inclusion criteria. Clinicopathologic findings included decreased mean albumin, packed cell volume and serum sodium were noted in addition to an increased mean serum creatine kinase at time of admission. Seven camelids (46.7%) survived to time of discharge. Eight cases received postmortem examinations, and the most significant findings were mild to severe skeletal muscle necrosis and minimal to mild spinal axonal degeneration.

Camelid heat stress should be considered as a primary or secondary disease process in a recumbent camelid with a history of suspected hyperthermia. Further research is needed to determine risk factors and response to treatments.

**ABSTRACT FA-12**
**COMPARISON OF THREE IMMUNOGLOBULIN G (IgG) ASSAYS FOR DIAGNOSIS OF FAILURE OF PASSIVE TRANSFER IN NEONATAL ALPACAS.** T Pinn[1], T Stokol[1], LF Gagliardo[2], SR Purdy[3], JA Appleton[2]. [1]Cornell University College of Veterinary Medicine, Ithaca, NY, [2]James A. Baker Institute for Animal Health, Cornell University, Ithaca, NY, [3]University of Massachusetts Amherst, Hadley, MA

Measurement of serum IgG is used for assessment of passive transfer of immunity in neonatal crias. While an IgG concentration below 1000 mg/dL is suggestive of failure of passive transfer (FPT) in Peruvian herds, this cut-off remains undefined in United States herds. The purpose of this study was to determine whether three commercially available assays yielded comparable results for IgG in crias. Serum samples from 91 crias submitted to Clinical Pathology for routine IgG measurement were used. Samples were stored frozen until batch analysis on the same day with the three assays. IgG was measured by radial immunodiffusion (assay A) and 2 immunoturbidimetric methods - one is configured for automated chemistry analyzers (assay B) and the other is a farm-side test (assay C). Median IgG concentrations were significantly different between the three assays. Assays A and B, which used the same standard, yielded higher IgG values than assay C,

resulting in a lack of concordance in 7-18% of samples at 1000 mg/dL IgG. Protein electrophoresis revealed that assay A and B standards contained mostly albumin (>60%), whereas assay C standard consisted of beta and gamma globulins. Median results from assay B after calibration with the assay C standard were not significantly different from those of assay C and diagnostic concordance between these 2 assays improved. Our results indicate that camelid IgG results are highly dependent on the assay standard and are not directly comparable between assays, potentially resulting in under-diagnosis of FPT in some crias.

## ABSTRACT FA-13

**BIOAVAILABILITY AND PHARMACOKINETICS OF ORAL MELOXICAM IN LLAMAS.** AJ Kreuder[1], PJ Plummer[1], LW Wulf[1], JA Schleining[1], B KuKanich[2], LL Layman[1], JF Coetzee[1]. [1]Iowa State University College of Veterinary Medicine, Ames, IA, [2]Kansas State University College of Veterinary Medicine, Manhattan, KS

South American camelids in the United States have rapidly developed into an important agricultural industry in need of veterinary services. Pain management is challenging in camelids because there are no drugs currently approved by the Food and Drug Administration for use in these species. Dosage regimens used for many therapeutic drugs have been extrapolated from other ruminants; however, the pharmacokinetics in camelids may differ from those of other species. Studies investigating the pharmacokinetics, in camelids, of non-steroidal anti-inflammatory drugs considered to be cyclooxygenase-2 (COX-2) selective are deficient in the published literature.

The aim of this study was to evaluate the oral bioavailability and pharmacokinetics of meloxicam in llamas in comparison to intravenous (IV) meloxicam. Six adult llamas (121- 168kg) were administered either a 1mg/kg dose of oral or a 0.5mg/kg dose of IV meloxicam in a randomized cross-over design with an 11 day washout period between treatments. Plasma samples collected up to 96 hours post-administration were analyzed by high pressure liquid chromatography and mass spectrometry detection (HPLC-MS) followed by noncompartmental pharmacokinetic analysis (results summarized in Table 1 below). No adverse affects in the llamas were associated with either treatment modality.

The mean bioavailability (F) of oral meloxicam was 76% indicating excellent gastrointestinal absorption. Plasma meloxicam concentrations >0.2 μg/mL were maintained for up to 72 h after oral administration; >0.2 μg/mL is considered to be the concentration of meloxicam required for analgesic effects in other species such as the horse. These data suggest that a single dosage of oral meloxicam at 1mg/kg could potentially maintain therapeutic concentrations in plasma for up to 3 days in adult llamas.

**Table 1.** Pharmacokinetic parameters. Mean (minimum - maximum).

| | AUC | Clearance | C0 / $C_{MAX}$ | $T_{MAX}$ | T ½ | F |
|---|---|---|---|---|---|---|
| | (hr*μg/mL) | (mL/min/kg) | (μg/mL) | (hr) | (hr) | (%) |
| **IV (0.5 mg/kg)** | 43.96 (31.4-55.6) | 0.190 (0.15-0.26) | 6.163 (5.26-7.63) | – | 17.4 (16.2-20.7) | – |
| **PO (1.0 mg/kg)** | 68.35 (52.4-79.2) | 0.248 (0.21-0.32) | 1.314 (0.83-1.78) | 21.4 (12.0-24.0) | 22.7 (18.0-30.8) | 76 (48-92) |

## ABSTRACT FA-14

**FLORFENICOL PHARMACOKINETICS IN HEALTHY ADULT ALPACAS, EVALUATING TWO COMMERCIALLY AVAILABLE DRUG FORMULATIONS.** D Bedenice[1], MG Papich[2], K Thane[1], K Holmes[1]. [1]Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, [2]North Carolina State University, Raleigh, NC

Single-dose florfenicol pharmacokinetics were compared using 2 commercially available drug formulations (Nuflor®, NuflorGold®, Schering-Plough) in 6 healthy adult alpacas, administered at 40 mg/kg subcutaneously (s.c.) using a randomized, cross-over design. Subsequently, each formulation of florfenicol was injected s.c. every 48 hours for 10 doses (40 mg/kg) to evaluate long-term effects. Clinical parameters were monitored daily in addition to weekly hematological analyses. Plasma drug concentrations were obtained via high-performance liquid chromatography at 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 18, 24, 36, 48, 60, 72, 96 and 120 h post-injection for single-dose studies, and at 0, 2, 4, and 8 h after the last dose for multi-dose studies. The florfenicol plasma concentrations vs. time plots were analyzed using a non-compartmental analysis (Nuflor®) or 2 compartment model (NuflorGold®), and commercial software (Phoenix WinNonlin 6.0). All data are presented as mean ± standard deviation; hematological parameters were compared between time points using repeated measures ANOVA and paired samples t-test post hoc.

Maximum plasma florfenicol concentrations ($C_{max}$) were achieved rapidly following single dose injections. Comparative pharmacokinetic parameters are listed in Table 1 below:

Maximum plasma concentrations of 4.48±1.28 and 7.62±1.65 μg/mL were achieved at steady state for Nuflor® and NuflorGold®, respectively, after 10 doses. The following hematological parameters decreased significantly between weeks 0 and 3, following repeated Nuflor® injection: total protein [TP] (6.38 vs. 5.61 g/dL, $P < 0.0001$), globulin (2.76 vs. 2.16g/dL, $P < 0.0003$), albumin (3.61 vs. 3.48g/dL, $P = 0.0038$), white blood cell count [WBC] (11.89 vs. 9.66×10^3/μL, $P < 0.044$), and hematocrit (27.25 vs. 24.88%, $P < 0.0349$). Significant clinical illness was observed in one alpaca. Similarly, the following parameters significantly decreased after 10 doses of 40mg/kg NuflorGold®: TP (6.65 vs. 5.98 g/dL, $P = 0.009$), globulin (2.82 vs. 2.28g/dL, $P = 0.004$), WBC (14.75 vs. 9.27×10^3/μL, $P = 0.001$). No clinical illness was observed in the latter group.

In conclusion, both Nuflor® and NuflorGold® may be erratically absorbed following s.c. administration in adult alpacas, based on the observed variability in most measured pharmacokinetic parameters. Plasma concentrations of NuflorGold® reached higher therapeutic concentrations, yet were associated with fewer systemic complications following long term use.

## ABSTRACT FA-15

**EVALUATION OF POINT OF CARE GLUCOSE METERS IN ALPACAS.** O Beemer, S Byers, A Bohn. Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO

Hospitalized alpacas are often hyperglycemic requiring repeated blood sampling to monitor glucose levels and response

**Abstract FA-14: Table**

| 40mg/kg s.c. | $C_{max}$ (μg/mL) | Time to $C_{max}$ (h) | AUC (h*μg/mL) | $T_{1/2}$ (h) | $T_{1/2}$ α (h) | $T_{1/2}$ β (h) |
|---|---|---|---|---|---|---|
| Nuflor® | 1.95±0.94 | 2.50±1.07 | 99.78±23.58 | 99.7±59.9 | - | - |
| NuflorGold® | 7.54±3.62 | 2.81±1.21 | 125.19±38.17 | - | 3.4±1.6 | 41.6±21.9 |

AUC – Area under the curve; $T_{1/2}$ (1 compartment) – half life; $T_{1/2}$ α and β (2 compartment)

to insulin therapy. Point-of-care (POC) glucometers are commonly used to measure whole blood glucose concentrations due to the rapid results and low expense. There is one POC glucometer currently marketed for use in animals; however this meter has not been validated for use in alpacas. Therefore human meters are used but also have not been validated or compared to clinical pathology laboratory glucose values for alpacas. Potential sources of variation include meter accuracy, hematological variations, and drug interactions. The purpose of this study was to compare 3 commercial glucometers to laboratory glucose readings.

Four alpacas were used in this randomized cross-over study. The alpacas were given either 0.4 U/kg of regular insulin intravenously or 500 mg/kg of dextrose IV with a 1 week washout between crossover periods. Blood samples were obtained from a jugular catheter at specific time periods starting at 10 minutes before drug administration until 6 hours post administration. Whole blood and plasma samples were measured on 3 glucometers, and serum glucose was measured on a laboratory chemistry analyzer.

The POC meters had variable results compared to the serum glucose and results also varied based on testing plasma or whole blood. All 3 meters had either proportional or constant bias but the variations were not considered clinically relevant. The POC meters can be used for alpacas but if more accurate blood glucose values are desired, reference intervals should be established.

**ABSTRACT FA-16**

**RISK FACTORS AND PROGNOSTIC VARIABLES FOR SURVIVAL IN 61 SICK NEONATAL NEW WORLD CAMELIDS.** FR Bertin, JM Squire, JE Sojka-Kritchevsky, SD Taylor. Purdue University, West Lafayette, IN

Neonatal New World Camelids (NWC) are more common among large animal patients, but information pertaining to clinical presentation and outcome is limited. The objective of this retrospective study was to describe the signalment, clinical signs, clinicopathologic values, antimicrobial use, and survival status in neonatal NWC presented to Purdue University Veterinary Teaching Hospital, and to identify variables for prediction of survival. Inclusion criteria were NWC less than 30 days of age with complete medical records that presented between 2000 and 2010. Cases were categorized by outcome, and groups were compared.

Sixty-one patients met the inclusion criteria (36 alpacas and 25 llamas). The median age at presentation was 1 day. There was a significant overrepresentation of females (69%, $P < 0.01$). The most common clinical findings included clinical sepsis (49%), failure of transfer of passive immunity (41%), and congenital defects (41%). Ophthalmologic (21%), gastrointestinal (15%), musculoskeletal (11%), respiratory (10%) and neurologic (10%) abnormalities were also reported. The most commonly administered antimicrobials were penicillin G (39%), trimethoprim-sulfadiazine (26%), amikacin (21%) and ceftiofur (10%).

Sixty-four percent of NWC survived to discharge. Clinicopathologic findings on admission were variable and nonspecific but albumin and $TCO_2$ were significantly lower in nonsurvivors ($P < 0.05$). The only independent factors associated with survival were absence of congenital disorders ($P < 0.01$), administration of hyperimmune plasma ($P < 0.05$), and antimicrobial treatment with penicillin G ($P < 0.05$) or trimethoprim-sulfadiazine ($P < 0.05$).

We conclude that congenital disorders are common in NWC, and that hypoalbuminemia and congenital disorders were predictors of nonsurvival. Plasma administration and antimicrobial treatment were associated with survival.

**ABSTRACT FA-17**

**EVALUATION OF THE USE OF INFRARED THERMOGRAPHY IN THE NASAL CANTHUS OF THE EYES OF HOLSTEIN CALVES WITH OR WITHOUT CLINICAL SIGNS OF ENTERITIS.** FCG Morelli, TS Poló, LG Ávila, B Gerardi, FLF Feitosa, LCN Mendes, JR Peiró. FMVA – Univ Estadual Paulista (UNESP), Araçatuba, SP, Brazil

The purposes of this study were to establish the pattern of infrared temperature in the nasal canthus of the eyes of Holstein calves with or without clinical signs of enteritis between 0 and 7 days old; observe whether calves with clinical signs of enteritis have elevated temperature in the nasal canthus compared with the healthy calves, and determine the correlation between the temperature of nasal canthus of the eye and rectum temperature of calves with or without signs of enteritis. Twenty Holstein calves, from two dairy farms were divided into two groups: without (control group) and with clinical signs of enteritis (experimental group). The animals were subjected to measurement of temperature in the nasal canthus of both eyes by infrared thermography, and rectum temperature by a digital thermometer. The animals were evaluated at birth and 1, 3, 5 and 7 days after birth. The reference values for the nasal canthus of the left (LE) and right (RE) eye were established for each group. There was no difference in rectal temperature between groups ($P = 0.6190$). The temperature in the nasal canthus did not significantly change in the LE ($P = 0.3892$) or RE ($P = 0.2491$) of calves in the control or experimental groups over 7 days of evaluation. The correlation between the temperature of the nasal canthus of calves the rectal temperature was 0.0007. We conclude that the reference values for IRT in the nasal canthus of eyes of calves with or without clinical signs of enteritis were generated. The use of the nasal canthus of eyes of calves has a significant correlation with the rectal temperature. Thus, the temperature of the nasal canthus of eyes of calves might be used to evaluate the body temperature of Holstein calves.

**ABSTRACT FA-18**

**DEVELOPMENT, SAFETY, AND EFFICACY OF AN AUTOGENOUS VACCINE FOR *MYCOPLASMA OVIPNEMONIAE* IN DOMESTIC SHEEP.** JC Ziegler, TE Besser, KK Lahmers, GM Barrington. Washington State University College of Veterinary Medicine, Pullman, WA

Epizootic pneumonia is a major rate limiting factor in the reestablishment of bighorn sheep (BHS) populations in western North America. *Mycoplasma ovipneumoniae* (Movi), which is carried by domestic sheep (DS) as a normal upper respiratory tract commensal flora, is the primary epizootic agent that triggers epizootic pneumonia of bighorn sheep.

Our hypothesis is that interventions to reduce/eliminate Movi in DS will reduce transmission to BHS. The objective of this preliminary study was to evaluate the safety and immunogenicity of two subcutaneous Movi vaccine formulations in DS: 1) live Movi without adjuvant at 0 and 30 days, and 2) Movi in oil adjuvant at 0 and 30 days. Injection sites were monitored and samples were collected periodically for complete blood count, PCR, bELISA, and peripheral blood mononuclear cell lymphocyte stimulation assay. Biopsies of the injection sites were performed 14 days post-inoculation. Vaccination with live Movi without adjuvant yielded no adverse reactions locally or systemically. Vaccination with live Movi and oil adjuvant showed mild local inflammation and fibrosis, but no adverse reactions were noted systemically. Neither vaccine formulation showed evidence of immunogenicity.

The vaccine formulations examined in this study were demonstrated to be safe for use in DS, but didn't exhibit immunogenicity or protection in the sheep inoculated. Alternative approaches toward improving immunogenicity must be explored, such as utilizing a different adjuvant. Another safety study would need to be performed before efficacy testing in a larger population.

**ABSTRACT FA-19**

**TEMPERATURE EVALUATION OF SHEEP SUBMITTED TO EXPERIMENTAL ENDOTOXEMIA.** EM Panelli, B Gerardi, LG Avila, RF Araujo, MG Carvalho, JR Peiro, SHV Perri, LCN Mendes. DCCRA – FMVA – unesp – Univ Estadual Paulista, Brazil

The present study aimed to assess by thermometry and thermography, changes in temperature affected by endotoxemia in sheep experimentally induced by inoculation of LPS from E. coli (400 ng / kg body weight). For this, eight sheep were used, with two years of age, obtained from commercial Araçatuba region. Of the eight animals used, four (group 1) were inoculated with LPS (Escherichia coli 055: B5, Sigma, St. Louis, MO) at a dose of 400 ng / kg and four (group 2) were inoculated with 2 ml of saline 0.9% Sodium Chloride (NaCl). We conducted a physical examination of animals at the time of admission and data recorded on individual sheets. After the physical examination was performed thermographic examination on admission of animals and the 2, 4, 6, 12, 24, 36, 48 and 60 hours after administration of LPS or saline. We analyzed the following regions: Temperature Front (TF), temperature of the dorsum (TD), axillary temperature (AT), temperature of the external face of the thigh (FEC), temperature of the inner thigh (FIC) and temperature of the perineum (TP). We can conclude that thermography can be used to analyze the temperatures of sheep with endotoxemia, better results were obtained by the analysis of the regions: (TF) at times M6 and M60, (TD), M6, M12, M60, (TA), M12; (FEC), and M12 (TFIC), M12. While the non-contact thermometry was not effective in observing the temperature rise caused by endotoxin administration.

**ABSTRACT FA-20**

**ARSENIC TOXICOSIS IN A HERD OF CATTLE.** FR Bertin[1], LJ Baseler[2], SD Lenz[2], CR Wilson[2], JE Sojka-Kritchevsky[1], SD Taylor[1]. [1]Department of Veterinary Clinical Sciences Purdue University, West Lafayette, IN, [2]Comparative Pathobiology, Purdue University, West Lafayette, IN

Arsenic toxicosis is uncommon in cattle, and successful treatment has not been previously reported. The objective of this study was to describe the clinical signs, clinicopathologic indices, treatment, and survival status of six cows intoxicated with arsenic.

Cows ranged from 1 to 4 years of age. Cattle were exposed to burned wood previously treated with arsenic. The ashes contained more than 2,400ppm of arsenic by a colorimetric qualitative test. The most common clinical signs included ataxia and weakness (5/6; 83%), dehydration (4/6; 67%), diarrhea (4/6; 67%), respiratory distress (3/6; 50%) and sudden death (1/6; 17%). Clinicopathologic values on presentation revealed hemoconcentration, azotemia, hypocalcemia, hyperbilirubinemia and increased liver enzyme activity in all animals. A diagnosis of arsenic toxicosis was made based on detection of elevated arsenic concentrations in whole blood, urine or tissues using inductively coupled plasma mass spectroscopy. The median blood arsenic concentration for presenting cows was 677ppb [276-747ppb] (reference range: $<50$ppb). Supportive treatment included intravenous and oral fluids, oral mineral oil and oral activated charcoal. Oral and intravenous sodium thiosulfate was used as a specific antidote. After treatment, the median blood arsenic concentration was 463ppb [48-514ppb].

There was no association between severity of clinical signs, blood arsenic concentration and outcome. Thirty-three percent of cows survived and were healthy for up to two months after discharge. Necropsy of nonsurvivors revealed abomasal erosions and submucosal edema, segmental jejunal mucosal congestion, and urinary bladder mucosal petechiae. Sodium thiosulfate may be of limited use in affected cattle.

**ABSTRACT FA-22**

**ANALYTICAL VALIDATION OF A COMMERCIALLY AVAILABLE IMMUNOASSAY FOR THE MEASUREMENT OF SERUM FOLATE CONCENTRATIONS IN PIGS.** N Grützner[1], D Knabe[2], BD Lawhorn[3], B Dominguez[3], J Kauffold[4], JS Suchodolski[1], JM Steiner[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX,, [2]Department of Animal Science, Texas A&M University, College Station, TX, [3]Veterinary Large Animal Clinical Sciences, Texas A&M University, College Station, TX, [4]New Bolton Center, University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA

Folate (vitamin $B_9$) plays an important role in amino acid metabolism, and in RNA and DNA synthesis. Based on literature from human and veterinary medicine the majority of folate is absorbed in the proximal small intestine. In humans, cats, and dogs it has been shown that chronic and severe proximal small intestinal disease can cause folate deficiency and that small intestinal dysbiosis can lead to increased serum folate concentrations. An immunoassay labeled for the measurement of serum folate concentration in humans is routinely used in dogs and cats. In pigs, the role of serum folate has not yet been extensively investigated. The aim of this study was to analytically validate said immunoassay, labeled for use in humans, for the measurement of folate concentrations in serum samples from pigs and to determine serum folate concentrations in weaned pigs.

For the analytical validation of the assay (IMMULITE® 2000 folate immunoassay; Siemens, Deerfield, IL), 20 surplus porcine serum samples were used. Validation of the assay consisted of determination of dilutional parallelism, spiking recovery, and intra- and inter-assay variability. Additional surplus serum samples from 21 piglets from four litters owned by and housed at a Texas A&M University farm were obtained. Each piglet had been bled twice, the first time at weaning (21 days of age) and the second time 12 days later. To investigate results in comparison between age groups (time of weaning and post weaning), serum folate concentrations were compared using a Wilcoxon matched pairs test. Significance was set at $p < 0.05$.

Observed to expected ratios (O/E) for serial dilutions ranged from 78.4 to 98.3% (mean ±SD: 85.2 ±6.9%) for five different serum samples for dilutions of 1:4, 1:8, 1:16, and 1:32. O/E for spiking recovery ranged from 84.9 to 115.2% (mean ±SD: 98.5 ±8.6%) for five different serum samples that had been spiked with each other. Intra-assay coefficients of variation (%CV) for five different serum samples were 5.5, 6.5, 7.2, 7.4, and 8.7%, respectively. Inter-assay %CVs for five different serum samples were 5.0, 6.7, 9.6, 10.4, and 12.5%, respectively. Serum folate concentrations were significantly higher in piglets post weaning (median [range]: 45.0 [21.0–90.5] μg/L) compared to those at the time of weaning (median [range]: 17.2 [8.5–50.7] μg/L; $p < 0.0001$).

The IMMULITE® 2000 folate immunoassay labeled for use in humans is linear, accurate, precise, and reproducible for measurement of serum folate concentrations in pigs. This study also shows that piglets that differ in age by only 12 days have significantly different serum folate concentrations. Further investigations of the implications of differing serum folate concentrations in pigs at different stages of life are warranted.

# SMALL ANIMAL – GASTROENTEROLOGY

**GI-4**

**CONFOCAL ENDOMICROSCOPY ENABLES *IN VIVO* IDENTIFICATION OF GASTRIC HELICOBACTER-LIKE ORGANISMS IN DOGS.** M J Sharman[1], B Bacci[1], P Sutton[2], T Whittem[1], P Delaney[3], C S Mansfield[1]. [1]School of Veterinary Medicine, The University of Melbourne, Werribee, VIC, Australia, [2]Centre for Animal Biotechnology, The University of Melbourne, Parkville, VIC, Australia, [3]Optiscan, Nottinghill, VIC, Australia

Confocal endomicroscopy (CEM) is an emerging technology allowing for *in vivo* cellular imaging of the gastrointestinal mucosa and is capable of achieving subcellular resolution. In

people CEM has been successfully used to identify and diagnose clinical *Helicobacter pylori* infections and associated gastritis. CEM has also been used to characterise various other gastrointestinal conditions including dynamic morphologic changes in the mucosa in response to provocative challenges. The objective of this study was to determine whether CEM could be used to identify *Helicobacter*-like organisms in dogs.

Six clinically healthy colony dogs underwent standard upper gastroduodenoscopy followed by CEM. CEM-guided pinch biopsies were obtained for comparison. Two fluorophores previously evaluated clinically in people, and shown to be effective and safe in dogs in a previous study, were used to provide contrast. Fluorescein (10% solution, 15mg/kg) was first administered intravenously followed by topical acriflavine (0.05% solution) via an endoscopy spray catheter.

CEM provided high quality *in vivo* cellular and subcellular histologically-equivalent images for evaluation during gastroduodenoscopy. Application of topical acriflavine allowed identification of *Helicobacter*-like organisms in all six dogs. Organisms were most commonly identified within the gastric mucus layer, but were frequently present deeper within gastric pits. Distribution throughout the stomach was scattered and multi-focal. CEM findings were correlated with standard histopathologic evaluation, along with polymerase chain reaction (PCR), serum antibody concentration and culture. CEM provided an accurate semi-quantitative assessment of *Helicobacter*-like organisms.

Confocal endomicroscopy provides *in vivo* histology images and is capable of aiding identification of *Heliocbacter*-like organisms and interpretation of mucosal morphology by gastroenterologists during the procedure. This has implications for further investigating the relationship between these organisms and inflammatory change in dogs.

## ABSTRACT GI-7

## SERUM PEPSINOGEN A AS A PROGNOSTIC MARKER IN CANINE GASTRIC DILATATION AND VOLVULUS.

I Aroch[1], J Steiner[2], I Israeli[1], S Yudelevitch[1], Y Bruchim[1], G Segev[1]. [1]Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot, Israel, [2]GI Laboratory, Texas A&M University, College Station, Texas, USA

Pepsinogens are zymogens secreted mainly by gastric chief cells. In humans, their serum concentration reflects gastric mucosal morphology and function. Our aims were to characterize serum canine pepsinogen A (cPG-A) and lactate concentrations at presentation in dogs with gatric dialtation and volvulus (GDV), assess their relationship with surgical findings and mortality, and compare their prognostic usefulness. Sixty-six dogs confirmed with GDV at surgery were included in the study group. Blood samples collected prospectively at presentation were used to measure cPG-A and lactate concentrations. The reference interval (RI) for cPG-A was based on 79 healthy, fasted controls. The gastric wall status was assessed and assigned a score (GWDS) at surgery.

The cPG-A RI was set at 105-802 μg/L. The mortality rate was 22.7%. cPG-A was higher in GDV dogs (median 397μg/L, range 37-5410) compared to controls (median 304 μg/L, range 18-848) ($P = 0.07$). In dogs with GDV, cPG-A level was higher in non-survivors compared to survivors (median 746 μg/L, range 128-5409 versus median 346, range 36-1575, respectively; $P = 0.003$). There was a significant ($P = 0.007$) trend for increased cPG-A with an increase in GWDS. Receiver operator characteristics analysis of cPG-A as a predictor of death had an area under the curve (AUC) of 0.756 and performed better compared to lactate concentration (AUC 0.66).

cPG-A is often increased in canine GDV at presentation. Its level is positively associated with GWDS, and is significantly higher in non-survivors compared to survivors. cPG-A performed better as an outcome predictor compared to serum lactate.

## ABSTRACT GI-12

## FELINE EXOCRINE PANCREATIC INSUFFICIENCY: 150 CASES.

PG Xenoulis[1], P Wooff[2], DL Zoran[2], LC Doyal[1], K Wooten[1], WN Cutrone[1], JS Suchodolski[1], JM Steiner[1]. [1]Gastrointestinal Laboratory and, [2]Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX

Exocrine pancreatic insufficiency (EPI) is considered to be a rare condition in cats. However, with the introduction of the feline TLI (fTLI) assay, which is considered to be the gold standard test for the diagnosis of feline EPI, this condition is diagnosed with increasing frequency. Due to the small number of reported cases, the clinical presentation and response to therapy of cats with EPI have been poorly described. The purpose of the present study was to describe the signalment, clinical signs, concurrent diseases, and response to treatment of a large number of cats with EPI.

The Gastrointestinal Laboratory's database was searched over a 15-month period for cats with a serum fTLI concentration $\leq$ 8 μg/L, which is the currently recommended cut-off value for a diagnosis of feline EPI. The veterinarians who had submitted the samples were contacted and asked to fill out a questionnaire for each affected cat. Information gathered involved signalment, medical history, clinical signs, concurrent conditions, and treatment.

Questionnaires for 150 cats with EPI were completed. Breeds included DSH (94 cats), DLH (15), DMH (11), Maine Coon (7), British shorthair (6), Siamese (6), Ragdoll (3), Abyssinian (1), Balinese (1), Himalayan (1), and Savannah (1), while 4 cats were of mixed breed. The mean age of the cats was 8.1 years (SD: 4 years). Sixty-one cats were female (all neutered) and 89 were male (86 neutered). The median body condition score was 3/9 (range: 1/9 to 7/9). Ninety-two of 119 cats (77%) had hypocobalaminemia (median: 149 ng/L; range: 149-1,001 ng/L), and 83 of those cats (70%) had undetectable serum cobalamin concentrations ($<$149 ng/L). Fifty-six of 119 cats (47%) had increased and 6 cats (5%) had decreased serum folate concentrations (median: 21.1 μg/L; range: 3.9-121 μg/L). The median serum Spec fPL concentration was 0.9 μg/L (range: 0.2-4.8 μg/L). The most common clinical sign was weight loss (median weight loss: 1.41 kg; range: 40 grams to 6.82 kg), and was reported in 91% of cases. Other clinical signs included loose stools (62%), poor haircoat (50%), anorexia (45%), increased appetite (42%), depression (40%), watery diarrhea (28%), and vomiting (19%). Eighty-seven cats (58%) were reported to have concurrent diseases, the most common of which were inflammatory bowel disease (21%), diabetes mellitus (14%), pancreatitis (11%), and hepatic lipidosis (6%). One hundred and two cats were treated with enzyme replacement therapy using appropriate products and the treatment response was reported to be good in 66%, partial in 24%, and poor in 10% of cases.

In the present study, the most common clinical sign in cats with EPI was weight loss. A relatively large percentage of cats did not have diarrhea. The majority of cats evaluated had hypocobalaminemia. Also, the majority of cats responded well to enzyme replacement therapy.

## ABSTRACT GI-1

## CHARACTERIZATION OF FECAL DYSBIOSIS IN DOGS WITH CHRONIC ENTEROPATHIES AND ACUTE HEMORRHAGIC DIARRHEA.

ME Markel[1], N Berghoff[1], S Unterer[2], LM Oliveira-Barros[3], A Grellet[4], K Allenspach[5], L Toresson[6], J Barr[1], RM Heilmann[1], JF Garcia-Mazcorro[1], JM Steiner[1], N Luckschander-Zeller[7], JS Suchodolski[1]. [1]GI Laboratory, Texas A&M University, College Station, TX, USA; [2]Ludwig-Maximilians University, Munich, Germany; [3]University of São Paulo, São Paulo, Brazil; [4]Royal Canin France, Alfort, France; [5]Royal Veterinary College, London, United Kingdom; [6]Helsingborg Referral Animal Hospital, Helsingborg, Sweden; [7]University of Veterinary Medicine, Vienna, Austria

Recent 16S rRNA gene sequencing studies of the duodenal and fecal microbiota have revealed alterations in the abundance of specific bacterial groups in dogs with various gastrointestinal disorders (e.g., decreases in *Clostridium* clusters IV and XIVa

with concurrent increases in Proteobacteria). The aim of this study was to validate the results of previous sequencing studies by measuring the abundance of selected bacterial groups using quantitative real-time polymerase chain reaction (qPCR) assays in healthy dogs, dogs with chronic enteropathies (CE), and dogs with acute hemorrhagic diarrhea (AHD).

Fecal samples were collected from healthy dogs (n = 180), dogs with CE (n = 87), and dogs with AHD (n = 48). A qPCR panel targeting 11 bacterial groups was used for analysis of fecal microbiota at various phylogenetic levels (i.e., *Faecalibacterium* spp., *Turicibacter* spp., *Bifidobacterium* spp., *Lactobacillus* spp., *Streptococcus* spp., Ruminococcaceae, *C. perfringens*, *E. coli*, γ-Proteobacteria, Bacteroidetes, and Firmicutes). Differences in bacterial abundance among groups were evaluated using a Kruskal-Wallis test followed by a Dunn's post-test. Statistical significance was set at $p < 0.05$.

Significant differences in the abundance of the evaluated bacterial groups were observed for the disease groups when compared to the healthy dogs. *Faecalibacterium* spp., *Turicibacter* spp., and Ruminococcaceae were significantly decreased in CE and AHD ($p < 0.001$ for all). Bacteroidetes were significantly decreased in CE ($p < 0.001$), but not different in AHD ($p > 0.05$). *E. coli* and *C. perfringens* were significantly increased in CE ($p < 0.05$ and $p < 0.001$, respectively) and AHD ($p < 0.001$ and $p < 0.01$, respectively). *Bifidobacterium* spp. and γ-Proteobacteria were significantly increased in CE ($p < 0.05$ for both), but not different in dogs with AHD ($p > 0.05$ for both). *Lactobacillus* spp. and *Streptococcus* spp. were significantly increased in dogs with CE ($p < 0.01$ for both) and decreased in dogs with AHD ($p < 0.05$ and $p < 0.01$, respectively). There was no significant difference in the abundance of Firmicutes in dogs with CE or AHD compared to the healthy dogs ($p > 0.05$).

In conclusion, the here employed qPCR panel revealed a fecal dysbiosis in dogs with CE and AHD when compared to healthy dogs. These results are similar to recently reported findings using molecular sequencing approaches. Quantification of these bacterial groups by qPCR may be a useful adjunct for diagnosis or monitoring of gastrointestinal disease in dogs.

## ABSTRACT GI-2

**FINAL DIAGNOSES IN 136 DOGS WITH CHRONIC DIARRHEA.** M Volkmann[1], GT Fosgate[2], JM Steiner[3], B Kohn[1]. [1]Clinic of Small Animals, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany, [2]Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, [3]Gastrointestinal Laboratory, Texas A&M University, College Station, TX

Chronic persistent or intermittent diarrhea is common in dogs and many different underlying causes have to be considered. The objective of this study was to evaluate the final diagnoses of a large number of dogs with chronic diarrhea.

Medical records of 209 dogs with chronic diarrhea (with or without vomiting) presented to the Small Animal Clinic of the Berlin University between September 2009 and July 2011 were retrospectively reviewed. Dogs were included if a minimum workup including a CBC, plasma chemistry profile, and fecal parasitology (flotation and Giardia ELISA) had been performed. Further diagnostic tests included abdominal radiography and/or ultrasonography, serum cTLI, cobalamin, folic acid, cortisol, or cPLI concentrations, fecal microbiology, a therapeutic trial with an exclusion diet and/or gastroduodenoscopy and/or colonoscopy.

In 136/209 (65%) cases a final diagnosis was recorded. A primary enteropathy was diagnosed in 123/136 dogs (90%): food responsive enteropathy (64), idiopathic inflammatory bowel disease (15), antibiotic responsive enteropathy (11; 9 metronidazole, 2 tylosin), steroid responsive enteropathy (4; corticosteroid-responsive without histopathologic confirmation of intestinal inflammation), gastrointestinal parasites (15), lymphoma (5), protein losing enteropathy of unknown etiology (3), protothecosis (2), and leishmaniasis, colorectal adenocarcinoma, colonic intussusceptions and systemic vasculitis in one dog each. In 13 dogs a secondary enteropathy was diagnosed (10%): exocrine pancreatic insufficiency (4), pancreatitis (3), and in one dog each a pancreatic adenocarcinoma, hypoadrenocorticism, hypothyroidism, portosystemic shunt, renal insufficiency, and heart failure. 57/123 dogs (43%) finally diagnosed with a primary enteropathy received antibiotics (other than or in addition to metronidazole, tylosin) without identifying an obligate enteropathogen.

In this study food responsive enteropathies (47%) were the most important differential diagnoses in dogs with chronic diarrhea.

## ABSTRACT GI-3

**SERUM HOMOCYSTEINE CONCENTRATIONS IN CHINESE SHAR PEIS AND DOGS OF SIX OTHER BREEDS WITH COBALAMIN DEFICIENCY.** N Grützner, VR Rangachari, RM Heilmann, K Weber, JS Suchodolski, JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX

Cobalamin deficiency in the Chinese Shar Pei (Shar Pei) is suspected to be hereditary. In humans, various defects of intracellular cobalamin metabolism have been reported in several different studies. All these defects result in deficient function of one of the two main cobalamin-dependent enzymes, methylmalonyl-CoA mutase and methionine synthase, which may lead to methylmalonic aciduria and homocysteinuria. In humans, both disorders have been characterized with increased serum methylmalonic acid concentrations and increased serum homocysteine (HCT) concentrations, respectively. In dogs, a previous study showed that cobalamin-deficient Shar Peis have higher serum methylmalonic acid concentrations compared to normocobalaminemic Shar Peis and cobalamin-deficient dogs of 6 other breeds. The aim of this study was to evaluate serum HCT concentrations in Shar Peis and dogs of 6 other breeds with cobalamin deficiency.

Serum samples from 40 Shar Peis with an undetectable serum cobalamin concentration (< 150 ng/L), 28 normocobalaminemic Shar Peis, and 279 dogs of 6 other breeds that were tested at the Gastrointestinal Laboratory between 2008–2011 and had an undetectable serum cobalamin concentration were included in this study. Serum cobalamin concentrations (reference interval: 251–908 ng/L) and serum HCT concentrations were measured using an Immulite®2000 assay (Siemens, Deerfield, IL) and a gas chromatography-mass spectrometry method, respectively. A control interval for serum HCT concentrations was established from 35 healthy pet dogs. Serum HCT concentrations were compared among cobalamin-deficient dogs belonging to the 7 different breeds using a Kruskal-Wallis test with a Dunn's post test. A Fisher's exact test was used to evaluate if cobalamin deficiency in Shar Peis is associated with hyperhomocysteinemia ($p < 0.05$).

Serum HCT concentrations ranged from 5.2–25.9 μmol/L (median: 10.3 μmol/L) in healthy pet dogs and the control interval for serum HCT was established as 5.0−22.1 μmol/L. Serum HCT concentrations were significantly higher in cobalamin-deficient Shar Peis (median: 25.4 μmol/L) than in cobalamin-deficient German Shepherd dogs (median: 9.4 μmol/L; n = 96), Labrador Retrievers (median: 10.7 μmol/L; n = 83), Yorkshire Terriers (median: 3.8 μmol/L; n = 44), Boxers (median: 11.8 μmol/L; n = 21), Cocker Spaniels (median: 5.0 μmol/L; n = 20), and Beagles (median: 9.0 μmol/L; n = 15; $p < 0.0001$). Serum HCT concentrations in normocobalaminemic Shar Peis ranged from 8.6–51.9 μmol/L (median: 13.7 μmol/L), and cobalamin deficiency in Shar Peis was significantly associated with hyperhomocysteinemia ($p = 0.0009$).

In conclusion, cobalamin-deficient Shar Peis had higher serum HCT concentrations compared to cobalamin-deficient dogs of 6 other breeds and a higher frequency of hyperhomocysteinemia than normocobalaminemic Shar Peis. These findings suggest that the function of the cobalamin-dependent enzyme methionine synthase is impaired in cobalamin-deficient Shar Peis.

**ABSTRACT GI-14**

**COMPARISON OF *E. COLI* ASSOCIATED WITH GRANULOMATOUS COLITIS OF BOXER DOGS AND *E. COLI* FROM HEALTHY CONROLS.** B Dogan, M Volkmann, J Oh, A Schukken, S Klaessig, KW Simpson. College of Veterinary Medicine, Cornell University, Ithaca, NY

Granulomatous colitis (GC) of Boxer dogs is a breed specific inflammatory bowel disease associated with mucosally invasive *E. coli*. Three of four *E. coli* strains isolated from 2 Boxers with GC were similar to adherent and invasive *E. coli* (AIEC) linked to Crohn's disease, raising the possibility that AIEC are adapted to exploit an IBD susceptible host. It is unclear if AIEC arise from the resident microbiome or are transmissible bacteria that could pose a zoonotic risk. Our objectives were to determine the prevalence, relatedness and virulence traits of AIEC associated with GC and then to compare GC-associated *E. coli* with strains from healthy dogs.

*E. coli* were isolated via colonic biopsies of 20 Boxers with GC and rectal mucosal swabs of 17 clinically healthy dogs. 10 to 15 isolates from each dog were evaluated for differences in genotype by RAPD-PCR with representative strains evaluated for phylogroup (A, B1, B2, D), serotype, multi locus sequence type (MLST-7), virulence genes, invasion of Caco-2 intestinal epithelial cells and survival in J774 macrophage cells. Gentamicin resistant, *cnf*- or *stx*-positive strains were excluded from cell culture assays (and could therefore not be identified as invasive or persistent).

We isolated 45 different *E. coli* strains from GC (1-4 / dog, median 2) and 34 from healthy dogs (1-3 /dog, median 2). 10/18 (55%) GC dogs harbored AIEC strains; 15/35 (42%) GC strains were AIEC and showed no consistent association with phylogroup or serotype, and were not clonal: 10/15 (68%) belonged to either phylogroup B1 or D. GC AIEC strains commonly carried *gsp* (73%), *hcp* (73%), *virB* (40%), $lpfA_{154}$ (40%) and *fyuA* (33%) genes.

13/18 (72%) GC dogs harbored non-AIEC strains; 20/35 (58%) strains isolated from GC dogs were non-AIEC strains. 15/20 (75%) belonged to phylogroup A or B1, and no serotype predominated. GC-associated non-AIEC strains also commonly carried *gsp* (80%), *virB* (80%), $lpfA_{154}$ (50%), *hcp* (45%) and *fyuA* (35%) genes.

10/17 (59%) healthy dogs harbored invasive strains; 16/18 (89%) gentamicin-susceptible strains were invasive (persistence in J774 was not assessed). 13/16 (81%) invasive strains belonged to phylogroup B1 or D. Invasive strains from healthy dogs commonly carried *virB* (100%), $lpfA_{154}$ (63%), *gsp* (56%), *hcp* (50%), *ratA* (44%), and *kpsMII* (38%) genes. The 2 non-invasive strains carried *hcp* (100%) genes. 8/34 healthy dog strains (all phylogroup B2) had an O4:H56 serotype.

Our results reveal that mucosal *E. coli* isolated from GC are not always AIEC. GC dogs do not have a substantially different proportion of invasive *E. coli* strains than healthy dogs; these *E. coli* share similar phylogroup compositions, virulence genes, and have no predominant serotype. Non-AIEC *E. coli* from GC dogs carry virulence genes commonly found in their AIEC counterparts. Therefore, GC-associated AIEC are likely resident flora that, when presented with an opportunity, invade and persist in mucosa of susceptible dogs leading to the clinical syndrome of GC.

**ABSTRACT GI-5**

**FECAL S100A12 CONCENTRATIONS ARE INCREASED IN DOGS WITH INFLAMMATORY BOWEL DISEASE.** RM Heilmann[1], A Grellet[2], K Allenspach[3], P Lecoindre[4], MJ Day[5], F Procoli[3], N Grützner[1], JS Suchodolski[1], JM Steiner[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, [2]Royal Canin, Aimargues, France, [3]Royal Veterinary College, University of London, UK, [4]Clinique vétérinaire des Cérisioz, St Priest, France, [5]Division of Veterinary Pathology, University of Bristol, Langford, UK

Diagnosis and treatment of inflammatory bowel disease (IBD) in dogs can be challenging. Serological or fecal markers of the disease that correlate with clinical severity would be very useful for the clinician. To date, only one serum marker, C-reactive protein, has been shown to correlate with clinical activity of disease and has shown some clinical utility in monitoring treatment of these patients. Surrogate inflammatory markers that can be measured in feces, such as the concentration of fecal S100A12, are considered useful tools for the detection of active gastrointestinal inflammation in humans but have not been evaluated in canine IBD. The aim of this study was to measure fecal S100A12 concentrations in dogs with IBD and to correlate these concentrations to clinical and histological markers of disease severity.

Spot fecal samples were collected from 29 dogs with IBD (median age [range]: 4 [1-13] years, 17 [59%] males) and 70 healthy control dogs (median age [range]: 4 [0.8-15] years, 37 [53%] males). Fecal S100A12 concentrations were measured by an in-house ELISA, and were compared between dogs with IBD and healthy controls using a Wilcoxon rank sum test. A Spearman rank sum correlation coefficient ρ with Bonferroni correction ($p < 0.0071$) was used to assess the relationship of fecal S100A12 concentrations with clinical activity (using the CCECAI scoring system) and with endoscopic and histologic disease severity (using 4-point semi-quantitative grading systems with 0 = inactive, 1 = mild, 2 = moderate, and 3 = severe changes).

Fecal S100A12 concentrations were significantly higher in dogs with IBD (median [range]: 273 [5-110,400] ng/g) compared to healthy controls (median [range]: 9 [1-1,810] ng/g; $p < 0.0001$) and correlated with the severity of endoscopic disease in the duodenum ($\rho = 0.503$; $p = 0.0064$) but not in the stomach ($p = 0.9501$) and had a trend to correlate with the endoscopic disease activity in the colon ($\rho = 0.815$; $p = 0.0136$). Fecal S100A12 concentrations were not associated with the severity of histological changes in the stomach ($p = 0.7749$), duodenum ($p = 0.5517$), or colon ($p = 0.0574$), but tended to be higher (15,830 [19-110,400] ng/g *vs.* 187 [5-7,370] ng/g; $p = 0.0783$) if histology revealed a neutrophilic component of the inflammatory infiltrate ($n = 5$). Fecal S100A12 concentrations showed a trend towards correlation with the CCECAI score ($\rho = 0.384$; $p = 0.0400$).

This study showed that fecal canine S100A12 concentrations are increased in dogs with IBD compared to healthy dogs and correlate with endoscopic disease severity. Further studies are under way to evaluate the mucosal expression of S100A12 in dogs with IBD and to assess fecal S100A12 concentrations in response to treatment.

**ABSTRACT GI-6**

**HISTOPATHOLOGICAL, CLINICAL, ENDOSCOPIC, AND ULTRASOUND FEATURES OF DOGS WITH CHRONIC ENTEROPATHIES AND SMALL INTESTINAL CRYPT LESIONS.** K Stroda[1,2], N Wakamatsu[2], L Gaschen[1], M Kearney[2], F Gaschen[1]. [1]Department of Veterinary Clinical Sciences, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA, [2]Department of Pathobiological Sciences, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA

This study was designed to retrospectively define the significance of small intestinal crypt lesions in dogs with chronic idiopathic enteropathies (CE). We hypothesized that crypt lesions are associated with more severe clinical disease in canine CE.

Dogs were selected from the LSU SVM biopsy database over a 5.5-year period. Histology slides were graded using the WSAVA system that ranks inflammatory and architectural mucosal lesions in the intestine. They were subsequently divided into those with (CL) and without crypt lesions (NC). CL were further differentiated in crypt abscesses (CA) and dilated crypts containing mucus (DC). Historical data, clinical signs and laboratory parameters were retrieved from the medical records. Duodenoscopy recordings were reviewed when available and graded using an established endoscopic scoring system. Additionally, abdominal ultrasound (AUS) exams were evaluated using a previously published scoring system. Finally, survival data was collected for all dogs. Data was statistically analyzed with ANOVA, Tukey's post hoc test, Kruskal-Wallis, and Wilcoxon Rank Sum as appropriate. Survival data was analyzed with Kaplan-Meier statistics.

Fifty-eight cases with idiopathic CE were found to have histopathologic duodenal lesions. They were divided as follows; 22 had CA, 9 had DC, while 27 had NC. The following paragraph summarizes the statistically significant differences ($p < 0.05$) detected between the 3 groups. Yorkshire terriers were significantly more

likely to have CA than other breeds. Dogs with CA and DC presented more frequently with ascites. When compared to NC and DC, dogs with CA were older (mean±SD, 96±35 months old vs. 59±40 and 51±33 months old) and had lower serum albumin (1.9±0.7 g/dL vs. 3.0±0.7 and 2.9±0.5 resp.), globulin (2.2±0.7 g/dL vs. 2.8±0.4 and 2.7±0.6 resp.) and total calcium (8.0±1.8 mg/dL vs. 9.6±0.6 and 9.5±0.8 resp.). Their small intestinal mucosal echogenicity scores and total AUS scores were greater, and they had more secondary AUS changes such as ascites. Additionally, mucosal edema was more frequently observed during duodenoscopy. Finally, histopathologic lacteal dilation and villous stunting were more frequent and more severe in dogs with CA than in those of other groups. Crypt necrosis and attenuation were more pronounced in dogs with CA than in those with DC. However, the severity and type of the inflammatory lamina propria infiltrates were not different between the three groups. The median survival of dogs with CA was 489 days and was significantly shorter than that of dogs with NC and DC, which could not be calculated.

Overall, the presence of CA is associated with more severe intestinal protein loss, AUS changes, mucosal architectural lesions, clinical course, and shorter survival in dogs with CE.

## ABSTRACT GI-16

## FELINE ALIMENTARY LYMPHOMA IS ASSOCIATED WITH CHANGES IN THE SPATIAL DISTRIBUTION OF MUCOSAL BACTERIA.

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Persistent bacterial infections are causally linked to gastric carcinoma and MALT-lymphoma in people, and the concept that mucosa-associated bacteria can promote gastrointestinal carcinogenesis is well established. Perturbation of the intestinal microbiome has been documented in cats with IBD and it seems plausible it could serve as a trigger for the development of intestinal lymphoma. It is against this background that we sought to assess the spatial distribution of mucosal bacteria in intestinal biopsies from cats with histologically normal small intestine, lympho-plasmacytic enteritis (LPE), and alimentary lymphoma.

Endoscopic or surgical biopsies of normal (N) small intestine (n = 16), LPE (n = 25), and alimentary lymphoma (small cell (SC), low grade, n = 33; large cell (LC), high grade, n = 16) were identified in the histopathology database at Cornell. The spatial distribution of mucosal bacteria (luminal cellular debris, villus-associated mucus, adherence to epithelium and serosa, mural invasion, intravascular presence) was determined by fluorescence in situ hybridization (FISH) with the eubacterial probe EUB338. The results of FISH analysis are shown in the table below:

The spatial distribution of mucosal bacteria was similar in N and LPE. Colonization of luminal cellular debris was more common in both SC and LC lymphoma than N and LPE. Bacterial colonization of adherent mucus was more common in SC lymphoma than LPE. Invasive bacteria were most frequently associated with lymphoma, particularly sections from the LC group. Bacterial invasion was not restricted to areas of eroded or ulcerated mucosa, and in some sections bacteria were visualized in vessels and/or the serosa.

Our results reveal that alimentary lymphoma is associated with alterations in the spatial distribution of mucosa-associated bacteria. Bacterial invasion was most frequent in LC lymphoma (69%), with evidence of bacterial translocation in approximately 30% of cases. Our results support the need to speciate lymphoma associated bacteria, and to evaluate cats with alimentary lymphoma for evidence of septicemia.

## ABSTRACT GI-8

## COMPARISON OF QUALITY AND DIAGNOSTIC ADEQUACY OF CANINE DUODENAL MUCOSAL BIOPSY SPECIMENS OBTAINED WITH DIFFERENT ENDOSCOPIC FORCEPS IN 17 DOGS.

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Limited published veterinary data exist regarding the influence of different flexible endoscopic forceps on canine duodenal mucosa biopsy quality and diagnostic adequacy.

With the hypothesis that larger forceps would procure superior biopsy specimens, we prospectively evaluated the effect of 6 different disposable biopsy forceps on duodenal biopsy weight, depth, crush artifact and assessment of histologic adequacy in 17 healthy adult dogs. Two operators each obtained 4 duodenal biopsy specimens from each dog with each forceps. The lightest sample was discarded and the 3 other specimens were blindly evaluated by one pathologist. A total of 612 specimens were evaluated. Results were analyzed by one-way ANOVA of forceps effects with dog as a blocking factor. Post hoc pairwise comparisons were examined with Tukey's test when indicated.

The operator had no statistically significant effect on any of the variables. Biopsies performed with the 2 Boston Scientific (BoS) Radial Jaw Large Capacity forceps were significantly heavier and their diagnostic adequacy was significantly superior to the Olympus Standard Oval and 'pediatric' forceps. However, no statistically significant difference in depth scores was noted. The BoS spike forceps was significantly associated with less crush artifact than the other forceps. The results also showed that the presence of a spike had no effect on crush artifact and diagnostic adequacy.

Overall, the two BoS Radial Jaw Large Capacity forceps were superior to the standard size forceps, demonstrating optimal quality and diagnostic adequacy. Our study provides valuable initial data to guide the choice of endoscopic biopsy forceps for duodenal samples.

## ABSTRACT GI-9

## SEROLOGIC AND FECAL MARKERS IN PREDICTION OF ACUTE DISEASE COURSE IN CANINE CHRONIC ENTEROPATHIES.

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Canine chronic enteropathies (CE) include a group of disorders characterized by persistent or recurrent gastrointestinal signs and histological intestinal inflammation. The search for biologic markers that can detect disease activity and monitor the effect of therapy has become an important focus of human CE research. Little information is available on pathologic markers that assess the disease course in dogs with CE, especially in response to diet and drug interventions. The purpose of this study was to evaluate the relationships between disease activity and serum and fecal biomarkers in dogs with CE.

Fifteen dogs with CE were prospectively enrolled. CE was diagnosed on the basis of chronic GI signs, the exclusion of identifiable underlying disorders, histological inflammation, and response to sequential therapy with an elimination diet, antibiotics, or immunosuppressive drugs. The clinical scores (i.e., CIBDAI), serum concentrations of C-reactive protein (CRP by

**Abstract GI-16: Table**

| | n | cell debris n(%) | mucus n(%) | epithelial n(%) | invasive n(%) | serosal n(%) | vascular n(%) |
|---|---|---|---|---|---|---|---|
| N | 16 | 7(44) | 2(13) | 3(19) | 1(6) | 1(6) | 0(0) |
| LPE | 25 | 13(52) | 2(8) | 6(24) | 0(0) | ND | ND |
| SC | 33 | 27(82)$^{**, \#}$ | 12(36)$^{\#}$ | 14(42) | 6(18)$^{\#}$ | ND | ND |
| LC | 16 | 16(100)$^{***, \#\#\#}$ | 3(19) | 6(38) | 11(69)$^{***, \#\#\#\#, \hat{}\hat{}}$ | 6(38) | 5(31)$^{*}$ |

Fisher's Exact Test, vs. N, $P < 0.05$ = *, $P < 0.01$ = **, $P < 0.001$ = ***; vs. LPE, $P < 0.05$ = #, $P < 0.001$ = ###, $P < 0.0001$ = ####; vs. SC, $P < 0.01$ = ^^.

ELISA), perinuclear antineutrophil cytoplasmic antibodies (p-ANCA by IFA), and canine calprotectin (cCP by ELISA) and fecal concentration of cCP were measured in each dog before and after 21 days of medical therapy. The correlation between CIBDAI with serum and fecal markers was estimated using the Spearman rank correlation coefficient. *P*-values $< 0.05$ were considered significant.

The final diagnoses for CE dogs included PLE (n = 7), idiopathic IBD (n = 5), antibiotic-responsive enteropathy (ARE; n = 2) and exocrine pancreatic insufficiency (EPI) with lymphoplasmacytic enteritis (n = 1). Treatment for CE resulted in full or partial remission in 14/15 dogs. Serum CRP ($P < 0.01$), fecal cCP ($P < 0.01$), and CIBDAI scores ($P < 0.01$) were significantly increased in CE dogs pre-treatment versus post-treatment values. There were no significant associations between the CIBDAI scores and pre- or post-treatment serum concentrations of CRP or p-ANCA. In contrast, there was a significant association ($P = 0.02$) between pre-treatment fecal cCP and CIBDAI scores. There was a trend toward higher pre-treatment serum CRP and fecal cCP concentrations to be found in dogs with moderate-to-severe disease activity (i.e., CIBDAI $\geq$ 6).

Our data indicate that fecal cCP levels were related to the extent of disease activity at diagnosis in dogs with CE. Serum and fecal biomarker concentrations are not associated with clinical disease activity in response to short-term medical treatment. Overall, dogs with severe GI disease more often have abnormal inflammatory markers, compared with dogs having low grade inflammation.

## ABSTRACT GI-10

### FLOW CYTOMETRICAL ANALYSIS OF PERIPHERAL BLOOD MONONUCLEAR CELLS IN DOGS WITH INFLAMMATORY BOWEL DISEASE.

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Inflammatory bowel disease (IBD) is a common cause of chronic gastrointestinal signs in dogs. Although the importance of intestinal intraepithelial lymphocytes (IEL) in the pathogenesis of canine IBD is evident, little is known about the role of peripheral blood mononuclear cells (PBMC) in this disease. Thus the purpose of this study was 1) to analyze PBMCs in canine patients suffering from IBD by flow cytometry (FCM) and 2) to compare PBMC sub-populations between patients and healthy controls.

Blood samples of 5 dogs suffering from IBD, which were clinically scored based on the canine IBD activity index (CIBDAI) and the canine chronic enteropathy clinical activity index (CCECAI) were collected. Blood samples of 10 healthy dogs served as controls.

PBMCs were isolated and stained with a panel of anti-canine and cross-reactive monoclonal antibodies against T and B cell differentiation antigens, including CD45, CD3, CD4, CD8α, CD8β, TCRαβ, TCRγδ, CD79 and CD21. All analyses were performed with multi-colour FCM.

Data of healthy dogs and dogs with IBD were compared by a Mann-Whitney U test. P values less than 0.05 were accepted as statistically significant.

Diseased dogs were suffering from moderate to severe IBD (CIBDAI: 6-11, median 9; CCECAI: 6-16, median 10). In canine IBD patients PBMCs showed significantly decreased percentages of CD79+ B-lymphocytes (p = 0.019), TCRγδ+ bearing T-lymphocytes (p = 0.03), including CD8α+ (p = 0.04) and CD8β+ (p = 0.02) T-cells, as compared to healthy dogs. The percentage of CD4+TCRαβ+ lymphocytes (p = 0.049) was significantly increased in dogs with IBD.

In summary, preliminary results demonstrate significant differences between PBMC populations in healthy and IBD dogs analyzed by FCM. These data suggest that canine IBD is associated with an altered distribution in PBMC populations, indicating an imbalance of lymphocytes with different immunological functions emphasizing the value of this examination technique in a larger cohort of dogs.

## ABSTRACT GI-11

### DECREASED IMMUNOGLOBULIN A LEVELS IN FECES, DUODENUM, AND PERIPHERAL BLOOD LYMPHOCYTES OF DOGS WITH INFLAMMATORY BOWEL DISEASE.

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Decreased immunoglobulin A (IgA) levels in fecal and duodenal explants were reported in German shepherd dogs (GSDs). This decrease in IgA levels was considered to be significant in the pathogenesis of inflammatory bowel disease (IBD). The aim of this study was to determine IgA levels in dogs of other breeds (non-GSDs) who had IBD.

Twenty healthy dogs and 21 dogs with IBD were used. IgA and IgG concentrations in serum, fecal, and duodenal samples were measured by ELISA. Duodenal lamina propria $IgA^+$ cells and $CD21^+$ $IgA^+$ cells in peripheral blood mononuclear cells (PBMCs) were examined by immunohistochemistry and flow cytometry, respectively.

Compared to the healthy dogs, IgA levels in the fecal ($P = 0.0053$) and duodenal ($P = 0.0233$) samples obtained from dogs with IBD were significantly reduced; however, no significant difference was observed in the IgG concentrations between the 2 groups. Furthermore, there was no significant difference in the serum IgA and IgG concentrations between the 2 groups. The number of duodenal $IgA^+$ cells was significantly decreased in dogs with IBD than in the healthy controls ($P < 0.0001$). Moreover, the proportions of $CD21^+$ $IgA^+$ cells in PBMCs were significantly decreased in dogs with IBD ($P < 0.0001$), and the proportions were significantly elevated in post-treatment compared to pre-treatment ($P = 0.0165$). In conclusion, decreased IgA levels in the feces, duodenum, and PBMCs were observed in non-GSDs with IBD, suggesting that the decreased IgA may be involved in the pathogenesis of canine IBD, regardless of the dog's breed.

## ABSTRACT GI-15

### GRANULOMATOUS COLITIS IN FRENCH BULLDOGS IS ASSOCIATED WITH INVASIVE *E. COLI* AND CLINICAL RESPONSE TO FLUOROQUINOLONE ANTIBIOTICS.

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French bulldogs have been reported to develop a form of inflammatory bowel disease that is histopathologically similar to granulomatous colitis of Boxer dogs (GCB). GCB is associated with mucosally invasive *E. coli,* whose eradication correlates with clinical remission. We sought to determine the clinical features, presence or absence of intramucosal bacteria and *E. coli* in colonic biopsies, and response to fluoroquinolone antibiotics in French bulldogs with GC.

Five French bulldogs (4M, 1F; median age 10mo, range 5-12mo) with a histological diagnosis of GC were studied. Bacterial colonization was evaluated using eubacterial (EUB-338) and *E. coli*-specific FISH probes. *E. coli* were isolated and antimicrobial resistance was determined by broth microdilution MIC from available fresh biopsies. Response to fluoroquinolone antibiotics was determined by monitoring clinical signs.

Dogs were presented with clinical signs of chronic hematochezia (5/5), large bowel diarrhea (3/5), and tenesmus (1/5) after failed therapeutic trials with metronidazole (5/5), various other antimicrobials (3/5), and anthelmintics. Age at onset of clinical signs was 1-7mo (median 3mo). Clinicopathologic findings and fecal analysis were unremarkable apart from mild anemia (PCV = 39%) in 1/5 dogs. Abdominal ultrasound revealed patchy thickening of the colonic wall and regional lymphadenopathy in 3/4 dogs. Multiple hypoechoic foci within the colonic submucosa were observed in 1/4 dogs. Colonoscopic findings

included hyperemic and irregularly thickened mucosa (5/5), overt bleeding (2/5) and mucosal ulceration (3/5). FISH revealed multifocal accumulations of intramucosal *E. coli* in colonic biopsies from 5/5 dogs. *E. coli* (2-6 strains) isolated from 2/2 dogs were susceptible to enrofloxacin, marbofloxacin and trimithoprim sulfa. Treatment with fluoroquinolones (enrofloxacin 4/5, marbofloxacin 1/5) at 4.4-10 mg/kg (median 10 mg/kg) PO SID for 6-10 weeks was associated with clinical remission. Hematochezia resolved in 3-14 days in 5/5 dogs, and 2 dogs had gained weight within 1 month. All 5 dogs remained free of clinical signs over a 9-23 month follow up period.

We conclude that GC in young French bulldogs is associated with the presence of invasive *E. coli* and closely parallels GCB. Treatment with fluoroquinolones was associated with lasting clinical remission. Documented treatment failures in GCB caution against empirical antimicrobial therapy in French bulldogs with unconfirmed GC.

## ABSTRACT GI-13
## EXOGENOUS SIALIC ACID AND CYSTEINE PROTEASE INHIBITION BLOCK ADHERENCE OF *TRITRICHOMONAS FOETUS* TO THE INTESTINAL EPITHELIUM.

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*Tritrichomonas foetus* (Tf) is a flagellated protozoan that parasitizes the feline colon resulting in chronic foul-smelling diarrhea. Up to 30% of young purebred cats are infected and this disease is now recognized as pandemic. Only a single drug, with a narrow margin of safety, is effective for treatment. While the venereal pathogenicity of bovine Tf is attributed to adherence to uterovaginal epithelium, the pathogenesis of diarrhea in feline Tf infection is unknown. Aims of this study were to establish an *in vitro* model of Tf adhesion to intestinal epithelium and apply this model towards investigating Tf cytopathogenicity and putative pharmacological targets.

Confluent monolayers of porcine intestinal epithelial cells (IPEC-J2, TER $\geq$ 2,000Ω·4.67cm$^2$) were infected with axenic cultures of feline Tf that were labeled with [$^3$H]thymidine (4μCi/ml for 48hrs) or CFSE (30μM for 20min) and harvested at log-phase. The effect on Tf cytoadhesion of multiplicity of infection, duration of infection, viability of Tf, binding competition, sialic acid (*N*-acetylneuraminic acid, 2-50μM), cytoskeletal (cytochalasin B, 10.5μM; colchicine, 250μM) and protease inhibitors (E64, 0.3-0.6mM; ethylenediaminetetraacetic acid, 0.5mM, 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride, 1-4mM, phenylmethylsulfonylfluoride, 0.5-1.5mM, pepstatin A, 0.01-0.1mM) were quantified by liquid scintillation counting and immunofluorescence of adherent Tf. A minimum of four replicates was performed for each experiment. Data were analyzed using commercially available statistical software.

$^3$[H]thymidine and CFSE-labeled Tf cytoadhered to IPEC-J2 monolayers in a dose and time-dependent manner with reproducible adhesion achieved by infection with $20\times10^6$ Tf for 6hrs. Clinical isolates of feline Tf (n = 5) demonstrated significantly greater adhesion to intestinal epithelium than feline *Pentatrichomonas hominis*, the latter of which is a presumably nonpathogenic trichomonad. Adhesion of Tf required viable trophozoites but was independent of cytoskeletal activity. Based on competition binding experiments, adhesion of Tf was receptor-ligand dependent. Treatment of Tf with *N*-acetylneuraminic acid and cysteine protease specific inhibitors, but not other classes of protease inhibitors, significantly blocked Tf adherence to the intestinal epithelium.

Identification of specific receptor-ligand dependent mechanisms of Tf adherence that are ameliorated by exogenous sialic acid and cysteine protease inhibitors suggests the presence of key molecular targets for prevention of Tf adhesion and consequent cytopathogenicity. The developed model provides a valuable resource for investigating pathogenic mechanisms of feline Tf and identifying novel molecular targets for the treatment of feline trichomonosis.

## ABSTRACT GI-17
## NATURALLY-OCCURRING HYPERADRENOCORTICISM IS ASSOCIATED WITH INCREASED PANCREATIC LIPASE IMMUNOREACTIVITY CONCENTRATIONS IN DOGS.

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Canine pancreatic specific lipase immunoreactivity concentrations (cPLI) are used to diagnose pancreatitis. The effect of naturally-occurring hyperadrenocorticism (HAC) on cPLI concentrations is unknown. The purpose of this study was to determine whether dogs with HAC have different cPLI concentrations by Spec cPL (SpeccPL) or SNAP cPL (SNAPcPL) assay from dogs without HAC.

Healthy dogs based on clinical examination and laboratory work (Group 1, n = 20), dogs with signs of HAC but normal ACTH stimulation tests (Group 2, n = 12), and dogs with HAC (Group 3, n = 20) were enrolled between December 2009 and November 2010. Dogs with signs compatible with pancreatitis were excluded. Samples were batched and SpeccPL and SNAPcPL assays were performed. SNAPcPL results were categorized as negative, low positive (LP), or high positive (HP). Associations between Group and cPLI were assessed using the Fisher's exact test (SNAPcPL) or Kruskal-Wallis test (SpeccPL). $P < 0.05$ was significant.

| | Cortisol (ng/ml) | | SNAPcPL | | | SpeccPL (mg/L) | | |
|---|---|---|---|---|---|---|---|---|
| | Pre-ACTH | Post-ACTH | Negative | LP | HP | < 200 | 200-400 | > 400 |
| Group 1 (n = 20) | 32 | 133 | 19 | 0 | 1 | 19 | 1 | 0 |
| Group 2 (n = 12) | 35 | 131 | 9 | 1 | 2 | 10 | 1 | 1 |
| Group 3 (n = 20) | 58 | 367 | 9 | 2 | 9 | 8 | 5 | 7 |

SNAPcPL results differed significantly between groups ($p = 0.005$), with more HP results in Group 3 than Group 1. SpeccPL concentrations were significantly higher in Group 3 than Group 1 or 2 ($p < 0.0001$).

Dogs with HAC had significantly higher SpeccPL concentrations and more HP SNAPcPL results than dogs without HAC. Further study is necessary to determine the clinical relevance of this association.

## ABSTRACT GI-18
## ALPHA-ENOLASE ANTIBODIES ARE NOT ASSOCIATED WITH FELINE PANCREATIC LIPASE IMMUNOREACTIVITY CONCENTRATIONS IN CLIENT-OWNED CATS.

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Alpha-enolase antibodies are a biomarker for inflammatory and autoimmune diseases in people. Pancreatitis is increasingly diagnosed in cats based on increased feline pancreatic lipase immunoreactivity (fPLI) concentrations. Histologically, it is characterized by lymphocytic infiltration, suggesting a potential underlying immune-mediated etiology. The purpose of this study was to evaluate for an association between alpha-enolase antibodies and fPLI concentrations in client-owned cats.

Residual sera were collected from 458 privately-owned cats following fPLI concentration measurement. Age was known for all cases. fPLI concentrations were classified as normal (2.0-6.8mg/l), questionable (6.9-12mg/l), or consistent with pancreatitis (> 12mg/l). Alpha-enolase antibody %ELISA values were deter-

mined using a previously validated assay and designated as positive or negative using a positive cutoff value of 80 based on results from 20 adult unvaccinated cats. A Fisher's exact test was used to assess for a relationship between fPLI category and α-enolase antibodies (%positive). Stepwise logistic regression analysis was performed with dichotomized alpha-enolase %ELISA results as the outcome variable. The final model included fPLI category, age, and the interaction between these factors. $P < 0.05$ was considered significant.

Antibodies against alpha-enolase were common. No significant associations were detected between alpha-enolase antibodies and fPLI concentrations.

| | Alpha-enolase antibodies | |
|---|---|---|
| fPLI category | % positive (n) | %ELISA mean (range) |
| Normal (n = 192) | 34.4 (66) | 74.60 (0–359.14) |
| Questionable (n = 108) | 27.8 (30) | 68.58 (0–317.35) |
| Positive (n = 191) | 24.1 (46) | 67.29 (0–631.94) |

The presence of alpha-enolase antibodies was not associated with fPLI concentrations. Prospective evaluation in cats with and without histologically confirmed pancreatitis should be considered to further assess for associations.

**ABSTRACT GI-19**
**MUCOSAL NUCLEOTIDE OLIGOMERIZATION DOMAIN TWO (NOD2) MRNA OVEREXPRESSION AND NF-KAPPAB ACTIVATION IN DOGS WITH CHRONIC ENTEROPATHY.** H Okanishi, K Hayashi, Y Sakamoto, T Watari. Nihon University, Kanagawa, Japan

Nucleotide oligomerization domain two (NOD2) is an intracellular receptor involved in the innate immune response against muramyl dipeptide, a component of the bacterial cell wall. Recently, NOD2 was reported to be overproduced in inflamed mucosa in Crohn's disease, and to be accompanied with a significant increase in NF-kappaB activity. However, few studies to date have investigated intercellular signal molecules in Chronic enteropathy dogs. The activities of NOD2 and NF-kappaB in mucosal biopsies from CE dogs, as compared to healthy controls, were investigated in the present study.

Endoscopic descending colon biopsies were obtained from 10 dogs referred for evaluation of chronic signs of large bowel disease. Biopsies from 5 healthy dogs were used as controls. Tissue was collected and immersed in liquid nitrogen (RNA) or formalin (histology). Expression of NOD2 mRNA was determined by RT-PCR, and was normalized against GAPDH. Amplified products were subjected to gel electrophoresis, followed by computer-assisted densitometry analysis using Quantity One Software. NF-kappaB binding activity was assessed by electrophoretic mobility shift assay.

The histological diagnosis was lymphocytic-plasmocytic colitis in CE dogs. NOD2 mRNA expression was significantly higher in CE dogs than in healthy controls ($p = 0.012$). Moreover, NF-kappaB binding activity was significantly upregulated in the inflamed colon mucosa of CE dogs, as compared to healthy controls ($p = 0.0047$).

In conclusion, NOD2 mRNA is overproduced and NF-kappaB activity is upregulated in the inflamed colon of CE dogs. Our data suggest that NOD2 and NF-kappaB play an important role in the pathogenesis of CE.

**ABSTRACT GI-20**
**FECAL AND URINARY N-METHYLHISTAMINE CONCENTRATIONS IN DOGS WITH CHRONIC GASTROINTESTINAL DISEASE.** N Berghoff, JS Suchodolski, JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX

The role of mast cells in dogs with chronic gastrointestinal (GI) disease is not well understood. Mast cells are potentially important in intestinal inflammation due to their ability to release inflammatory mediators, such as histamine. Histamine is rapidly metabolized upon its release, but one of its metabolites, N-methylhistamine (NMH), can be readily measured in both fecal and urine samples. The purpose of this study was to measure NMH in fecal and urine samples from dogs with chronic GI disease, and to evaluate its utility as a potential inflammatory marker in dogs.

Three consecutive fecal samples and one urine sample were collected from 53 dogs with chronic GI disease. Fecal and urinary NMH concentrations were measured by gas chromatography/mass spectrometry. Fecal NMH concentrations were reported as 3-day means and also as the maximum concentration for the 3 days. Urinary NMH was normalized using urinary creatinine concentrations and results were expressed as ng NMH/mg creatinine. Fecal NMH concentrations were compared to those in a group of 49 control dogs, and urinary NMH concentrations were compared to a previously established control range. A correlation between fecal and urinary NMH concentrations and the clinical IBD activity index (CCECAI) in dogs with GI disease was calculated using Spearman's rank correlation. Statistical significance was set at $p < 0.05$.

Mean 3-day fecal NMH concentrations were significantly higher in the dogs with GI disease (median: 97 ng/g, range: 0-4,690 ng/g) than in the control dogs (median: 53 ng/g, range: 9-252 ng/g; $p < 0.009$). Similarly, 3-day maximum NMH concentrations were significantly higher in the dogs with GI disease (median: 160 ng/g, range: 0-7,059 ng/g) than in the control dogs (median: 83 ng/g, range: 14-666.2 ng/g; $p < 0.01$). Eleven dogs (19%) had 3-day mean fecal NMH concentrations above the upper limit of the reference interval (>191 ng/g), and 23 dogs (36%) had maximum fecal NMH concentrations above the upper limit of the reference interval (>334 ng/g). Sixteen dogs (30%) had urinary NMH concentrations above the upper limit of the control range (>136 ng/mg creatinine), 8 of which also had simultaneously increased fecal NMH concentrations. There was no correlation between fecal and urinary NMH concentrations, or between these parameters and the clinical activity index.

These data show that fecal and/or urinary NMH concentrations may be increased in a subset of dogs with chronic GI disease. The lack of correlation to the clinical IBD activity index suggests that mast cell related inflammation may not be of clinical importance in all dogs with GI disease. The lack of correlation between fecal and urinary NMH concentrations may be due to a difference in distribution of mast cells within the mucosa, or may be due to potential uptake of NMH along the GI tract, leading to subsequent urinary excretion. Further studies are underway to evaluate the distribution of intramucosal mast cells and the correlation between fecal and urinary NMH concentrations and mucosal mast cell numbers in dogs with chronic GI disease.

**ABSTRACT GI-21**
**SERUM COBALAMIN AND METHYLMALONIC ACID CONCENTRATIONS IN DOGS WITH CHRONIC GASTROINTESTINAL DISEASE.** N Berghoff, JS Suchodolski, JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX

The prevalence of hypocobalaminemia in dogs with chronic GI disease has been reported to range from 6-18%. A recent study investigating cobalamin concentrations in canine serum samples submitted to the GI Laboratory has shown that 46% of dogs with hypocobalaminemia, and even dogs with low normal serum cobalamin concentrations may have evidence of cellular cobalamin deficiency, as evidenced by an increased serum methylmalonic acid (MMA) concentration. It is unknown how prevalent cobalamin deficiency on a cellular level is in dogs with chronic GI disease. Thus, the aim of this study was to determine the proportion of dogs with chronic GI disease that have hypocobalaminemia and/or methylmalonic acidemia.

Serum samples were collected from 58 dogs with chronic GI disease that had not previously been treated with cobalamin, and in which exocrine pancreatic insufficiency (EPI) had been excluded by measurement of serum trypsin-like immunoreactivity. Serum cobalamin and MMA concentrations were measured in all 58 dogs and compared to a group of 43 healthy control dogs using a Mann-Whitney U test. A correlation between serum cobalamin and MMA concentrations and the clinical IBD activity index (CCECAI) in dogs with chronic GI disease was evalu-

ated using Spearman's rank correlation. Statistical significance was set at $p < 0.05$.

Serum cobalamin concentrations were significantly lower in dogs with GI disease (median [range]: 334 [149-1,000] ng/L) than in control dogs (median [range]: 515 [333-835] ng/L; $p < 0.001$). Twenty-one dogs with GI disease (36%) had hypocobalaminemia (< 251 ng/L), and 8 dogs (14%) had undetectable cobalamin concentrations (< 150 ng/L). Serum MMA concentrations were significantly higher in dogs with GI disease (median [range]: 741 [442-205,399] nmol/L) than in control dogs (median [range]: 649 [228-1253] nmol/L; $p < 0.046$). Five dogs with GI disease (9%) had MMA concentrations above the reference interval (> 1,192.5 nmol/L). There was a significant negative correlation between serum cobalamin and MMA concentrations in dogs with GI disease (Spearman r = -0.435, $p < 0.001$). No correlation was found between the clinical activity index and serum cobalamin or MMA concentrations.

These data show a prevalence of 36% for hypocobalaminemia in dogs with chronic GI disease. Only 9% of these dogs also had increased serum MMA concentrations, suggesting cobalamin deficiency on a cellular level. This number is lower than previously reported, where 46% of dogs with a serum cobalamin concentration < 251 ng/L had increased MMA concentrations. However, sample populations between the two studies were likely different, because no clinical data was available for the dogs in the previous study, and dogs with neoplasia and EPI could not be excluded from analysis. This may have led to the difference in the results observed. In conclusion, serum cobalamin deficiency is commonly seen in dogs with chronic GI disease, but does not always appear to be associated with a deficiency of cobalamin on the cellular level.

## ABSTRACT GI-22

**SERUM CONCENTRATIONS OF CANINE ALPHA$_1$-PROTEINASE INHIBITOR IN YORKSHIRE TERRIERS WITH AND WITHOUT COBALAMIN DEFICIENCY.** N Grützner, RM Heilmann, JS Suchodolski, JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX

Protein-losing enteropathy (PLE) is a condition associated with an excessive loss of plasma proteins into the gastrointestinal tract. Important causes of PLE are an altered lymphatic drainage (e.g., intestinal lymphangiectasia) or an increased mucosal permeability (e.g., inflammatory bowel disease). Fecal canine alpha$_1$-proteinase inhibitor (c$\alpha_1$-PI) concentration has been reported to be increased in dogs with PLE due to its loss into the gastrointestinal tract, and a chronic loss of c$\alpha_1$-PI may theoretically deplete c$\alpha_1$-PI in serum, potentially altering the proteinase/-proteinase inhibitor balance. Serum c$\alpha_1$-PI concentrations, which during health are regulated within a narrow concentration range, have been reported to be decreased in a small group of dogs with PLE. PLE has been reported to occur frequently in certain dog breeds, one of these breeds being the Yorkshire Terrier. Also, it has been shown that inflammatory bowel disease and PLE in dogs is often associated with low serum cobalamin concentrations. The aim of this study was to compare serum concentrations of c$\alpha_1$-PI in Yorkshire Terriers with and without cobalamin deficiency.

Serum samples from 122 Yorkshire Terriers, which were submitted to the Gastrointestinal Laboratory between 2008 and 2011, were used retrospectively. Serum cobalamin concentrations (reference interval: 251–908 ng/L) were measured using an automated chemiluminescence assay (Immulite®2000; Siemens, Deerfield, IL). Serum c$\alpha_1$-PI concentrations (reference interval: 749.9–1,556.0 mg/L) were measured using an in-house radioimmunoassay. A Mann-Whitney *U* test was used to compare serum c$\alpha_1$-PI concentrations between Yorkshire Terriers with and without cobalamin deficiency. A $\chi^2$-test was used to evaluate if cobalamin deficiency in Yorkshire Terriers is associated with decreased serum c$\alpha_1$-PI concentrations. Significance was set at $p < 0.05$.

Fifty-three Yorkshire Terriers with an undetectable serum cobalamin concentration (< 150 ng/L) and 69 Yorkshire Terriers with a serum cobalamin concentration within the reference interval (median [range]: 618 [394–956] ng/L) were included. Serum concentrations of c$\alpha_1$-PI were significantly lower in cobalamin-deficient Yorkshire Terriers (median [range]: 1,027.5 [315.0–3,945.0] mg/L) compared to Yorkshire Terriers with a normal serum cobalamin concentration (median [range]: 1,665.0 [900.0–2,970.0] mg/L; $p < 0.0001$). One fourth of the cobalamin-deficient Yorkshire Terriers had a serum c$\alpha_1$-PI concentration below the lower limit of the reference interval (< 749.9 mg/L), and cobalamin deficiency was significantly associated with a decreased serum c$\alpha_1$-PI concentration ($p < 0.0001$).

In this study, serum concentrations of c$\alpha_1$-PI were decreased in cobalamin-deficient Yorkshire Terriers compared to normocobalaminemic Yorkshire Terriers. The functional and potentially prognostic implications of this decrease in serum c$\alpha_1$-PI concentrations warrant further studies.

## ABSTRACT GI-23

**EFFECTIVITY OF AST-120 IN DOGS WITH CHRONIC INFLAMMATORY ENTEROPATHIES.** M Volkmann[1], NC Wirtherle[2], GF Beddies[2], B Kohn[1]. [1]Clinic of Small Animals, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany, [2]Bayer Animal Health GmbH, D-51368 Leverkusen

Canine chronic inflammatory enteropathies (CIE) are highly complex diseases of unknown origin. Abnormal immunological tolerance towards environmental and luminal bacterial antigens might play an important role. AST-120 is a spherical carbon adsorbent preparation with a highly adsorption ability for low molecular substances.

The objective of this study was to evaluate the clinical efficacy of supplementary feeding of AST-120 in dogs with CIE.

Sixteen client-owned dogs with chronic diarrhea and no or insufficient response to an elimination diet were included into the explorative, randomized, placebo-controlled, double blinded field study. The dogs received either AST-120 (n = 8) or placebo (n = 8) for a duration of 21 days. The Canine Inflammatory Bowel Disease Activity Index (CIBDAI) was used to assess disease severity and clinical outcome. Remission was defined as complete (75% or greater reduction in CIBDAI score) or partial (< 75% and > 25% reduction).

A significant reduction in the mean CIBDAI score was observed in the AST-120 group (day 1: 4.6, day 21: 1.8; $p = 0.016$, Wilcoxon signed-rank test) but not in the placebo group (day 1: 4.4, day 21: 3.6; $p = 0.656$). After 21 days, 7/8 dogs treated with AST-120 (3 complete, 4 partial) and 3/8 dogs treated with placebo (2 complete, 1 partial) were in remission. An increase of body condition score was observed in 5/8 dogs treated with AST-120 but remained unchanged in the placebo group. No side effects of AST-120 were noted.

The data suggest that AST-120 may represent a safe and effective alternative therapy for dogs with mild to moderate CIE.

## ABSTRACT GI-24

**INTERLEUKIN-17A GENE EXPRESSION IN THE INTESTINAL MUCOSA IN DOGS WITH LYMPHOCYTIC-PLASMACYTIC ENTERITIS AND INFLAMMATORY COLORECTAL POLYP.** H Ohta, K Takada, Y Tamura, K Nakamura, M Yamasaki, M Takiguchi. Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan

Interleukin (IL)-17A is a proinflammatory cytokine produced by T helper (Th)17 cells, and an increase in IL-17A expression in the intestinal mucosa has been reported in human inflammatory bowel disease (IBD). Lymphocytic-plasmacytic enteritis (LPE) is the most common form of canine IBD, and IL-17A messenger RNA (mRNA) expression has not been examined in dogs with LPE. Inflammatory colorectal polyp (ICRP) in miniature dachshunds is recently recognized in Japan as a major cause of large bowel symptoms in this breed. ICRP is characterized by the formation of multiple small polyps (small polyps) in the large bowel mucosa, accompanied by a large space-occupying polyp (large polyp) formation, and is thought to be a novel form of IBD. The aim of the current study was to evaluate the mRNA expression of IL-17A in the duodenal mucosa from dogs with LPE and colorectal mucosa form dogs with ICRP.

Duodenal mucosae from 20 dogs with LPE were obtained by endoscopic biopsy. Endoscopic or surgical colorectal biopsies from 6 dogs with ICRP were obtained. Duodenal and colonic mucosae were obtained from 8 control dogs. Total RNA was extracted from intestinal mucosa and cDNA was synthesized. IL-

17A mRNA expression was measured by a quantitative reverse transcriptase polymerase chain reaction technique. In addition, mRNA expression of IFN-gamma (Th1 cytokine), IL-4 (Th2 cytokine) and IL-10 (immunoregulatory cytokine) was measured. Results were normalized using two housekeeper genes (GAPDH and SDHA).

The mRNA expression of all cytokines in the duodenal mucosa from dogs with LPE was not different from those of the control dogs. IL-17A, IFN-gamma, and IL-10 mRNA expression in large polyps, but not in small polyps, was significantly higher than that in colonic mucosa form control dogs. IL-4 mRNA expression was not different among groups. IL-17A mRNA expression in large polyps was negatively-correlate with IL-10 mRNA expression. In addition, IL-17A mRNA expression was strongly-correlate with serum C-reactive protein (CRP) concentration. Meanwhile, IL-10 mRNA expression was negatively-correlate with serum CRP concentration.

These findings indicated that the IL-17A mRNA expression was not increased in the duodenal mucosa in dogs with LPE. On the other hand, IL-17A mRNA expression was increased in large polyps in dogs with ICRP which composed mainly of neutrophils. IL-17A may play an important role in the recruitment of neutrophils and the formation of large polyps in colorectal area in dogs with ICRP, while IL-10 could oppose the proinflammatory effect of IL-17A.

## ABSTRACT GI-25

## C-REACTIVE PROTEIN CONCENTRATIONS IN DOGS WITH INFLAMMATORY BOWEL DISEASE AND CORRELATION WITH A CLINICAL DISEASE ACTIVITY INDEX AND SERUM ALBUMIN CONCENTRATIONS.

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C-reactive protein (CRP) is a positive acute-phase protein and has been considered as a useful biological marker in dogs with inflammatory bowel disease, particularly for monitoring the response to treatment. The canine inflammatory bowel disease activity index (CIBDAI) is used to assess clinical disease severity. The purpose of this study was to compare serum CRP concentrations between dogs diagnosed with inflammatory bowel disease (IBD) and healthy dogs, and to correlate serum CRP concentrations with CIBDAI scores and serum albumin concentrations in affected dogs.

Twenty dogs referred to the Small Animal Teaching Hospital at the School of Veterinary Medicine, University of São Paulo, Brazil, with a diagnosis of IBD were included in this prospective study. The severity of clinical disease was assessed using the CIBDAI scoring system. Blood samples were collected from these animals and from healthy control dogs (n = 20). Serum CRP concentrations were measured using a commercial ELISA kit (Tri-Delta Phase CRP Canine kit, Tri-Delta Diagnostic, Boonton Township, NJ). The data were statistically analyzed using GraphPad InStat version 3.10 for Microsoft Windows. Data were expressed as medians and range. The Mann-Whitney U test and Spearman's rank order correlation test were used. Statistical significance was set at $P < 0,05$ for all analyses.

Dogs in the IBD group had significantly higher serum CRP concentrations compared to dogs in the control group (medians and range – IBD group: 1.85 mg/L *versus* control group: 0.10 mg/L; P = 0.0244; Mann-Whitney *U* test). Serum albumin concentration in the IBD group was 3.20±0.67g/dL (mean ± standard deviation; reference interval: 2.5 – 4.5g/dL). Only one animal in IBD group had serum albumin concentration below the reference interval (< 2.5 g/dL) and all other animals had normal serum albumin concentration. No correlation was found between serum CRP and albumin concentrations(r = -0.098; P = 0.7475; Spearman correlation coefficient). The median CIBDAI score in affected dogs was 5 (range 0 to 9). A direct moderate positive correlation was found between serum CRP concentration and CIBDAI score (r = 0.613; P = 0.0040; Spearman correlation coefficient).

Hypoalbuminemia were not frequently observed in dogs with IBD. Elevated serum CRP concentrations of affected dogs suggest that this protein can be useful as a biological disease marker. Also, our findings indicate that CRP can reflect the severity of clinical disease. Only one animal had serum albumin concentration.

## ABSTRACT GI-26

## THE EFFECT OF A MULTI-STRAIN SYNBIOTIC ON THE MICROBIOTA OF CATS WITH CHRONIC ENTERITIS.

CB Webb[1], JF Garcia-Mazcorro[2], JM Steiner[2], JS Suchodolski[2]. [1]Colorado State University, Fort Collins, CO, [2]Gastrointestinal Laboratory, Texas A&M University, College Station, TX

Alteration of the host microbiota is one proposed mechanism for the potentially beneficial effect of pre- and probiotics in patients with diarrhea. The purpose of this study was to test the hypothesis that oral administration of a synbiotic (Proviable™-DC, Nutramax Laboratories) containing a total of $5x10^9$ CFUs of a mixture of multiple probiotic strains and a blend of prebiotics would significantly alter the microbiota of cats with chronic diarrhea.

Adult cats (n = 21) presenting for chronic diarrhea (≥ 3 months) without prior treatment were recruited and screened for systemic disease and parasitism. The cats were then administered the synbiotic once daily. Owners recorded stool quantity and consistency before and after supplementation. After 21 days cats underwent endoscopy for histopathology and characterization of the mucosa-adherent microbiota. One cat was found to have GI lymphoma and removed from the study. For analysis, cats were grouped into responders (R; n = 13) and non-responders (NR; n = 7) based on the subjective assessment of response to therapy by the owner. Mucosal biopsies (n = 13 and 7 for group R and NR, respectively) and paired pre- and post-synbiotic fecal samples (n = 5 each for groups R and NR) were used for microbiota analysis by 16S rRNA gene 454-pyrosequencing and qPCR assays. Differences in the abundance of bacterial groups and baseline characteristics between R and NR were assessed by Wilcoxon signed rank test or Kruskal-Wallis test. Differences in microbial community structure between R and NR and pre- post-synbiotic administration were assessed by the phylogenetic Unifrac distance method, followed by Analysis of similarity (ANOSIM) of the distance matrix. Significance was set as $p < 0.05$.

There were no significant differences in age, body weight, serum cobalamin or folate concentrations, duration of clinical signs, or fecal scores between the 2 groups prior to supplementation (p = 0.10). Histopathology revealed mild (n = 8), moderate (n = 9), or severe (n = 3) enteritis. No significant differences in microbiota composition of the mucosa-adherent microbiota were observed between R and NR after synbiotic use. However, in feces, the community structure at baseline differed significantly between R and NR (p = 0.04). The abundance of probiotic genera in feces at baseline was significantly higher in the NR than in R (p = 0.016). The NR displayed a significantly lower bacterial diversity (median Chao index: 130, range 86-169 vs. 306, range 186-313; p = 0.03) in feces. No differences in fecal microbial community structure were observed between pre- and post-synbiotic use samples.

In this study, synbiotic administration did not significantly alter the microbial community structure of cats regardless of their clinical response. The lower diversity of the microbiota, as well as the higher abundance of probiotic genera in the non-responder cats at baseline, may help to explain the failure of their diarrhea to improve with synbiotic use.

## ABSTRACT GI-27

## REGIONAL GRANULOMATOUS ENTERITIS IN 14 DOGS.

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Regional granulomatous enteritis is a rare form of canine inflammatory bowel disease (IBD) characterized by transmural granulomatous inflammation primarily involving the distal ileum and ileo-colic junction, and resulting in a stenosing, mass-like

thickening of the affected areas. The aim of this retrospective study is to describe the clinical and morphological characteristics of this condition, to describe and characterize the histological lesions observed and to report the treatments used and long-term follow-up.

Clinic data was reviewed to find dogs with a histopathological diagnosis of granulomatous enteritis.

Fourteen dogs were included in the study. All dogs were purebred (5 French Bulldog, 4 Yorkshire Terrier, 2 West Highland White Terrier, 2 Cavalier King Charles Spaniel, 1 English Cocker Spaniel), with 7 males and 7 females and a mean age of 6.6 years (range 2.5-13), body condition score was 3 to 4/9. All dogs presented with about 6 months history of chronic small intestinal diarrhea and weight loss. Four dogs had concurrent tenesmus, hematochezia, abdominal pain and vomiting. According to the clinical index CIBDAI score (Jergens), all dogs were scored 10 or more. In all dogs a firm, tubular soft mass was palpated in the cranial abdomen. Mild to moderate hypoproteinemia was present in 4 cases, and was severe in one case (total protein 3.3g/dl). On abdominal ultrasonography a segmental, parietal thickening of both the distal ileum and ileo-colic junction with loss of layering was seen. In 3 dogs there was an obstructive pattern and in 1 an intestinal intussusception was identified. Gastroduodenoscopy and colonoscopy was performed in 12 dogs and inflammatory lesions of the ascending colon were seen in 6 dogs. Exploratory coeliotomy performed in 9 dogs revealed: severe thickening of the ileum (n = 9), serosal lipogranulomas (n = 8), inflammation of the caecum and ileo-colic junction (n = 9) and inflammation of the ascending colon (n = 5). Histology of intestinal biopsies revealed a transmural granulomatous inflammation with voluminous lipogranulomas in the muscularis and serosa in all cases. In 9 dogs, an enterectomy was performed because of partial or total intestinal obstruction. Medical treatment with antibiotics (enrofloxacin and metronidazole) and immunosuppressive drugs (prednisolone and/or cyclosporine) were used in all dogs. A long-term (>6months) clinical improvement was seen in 7 cases, with a complete remission in 6.

Regional granulomatous enteritis seems to be a specific entity affecting small breed dogs, characterized by profound parietal changes in the intestine with preferential localization to the ileum and the ileo-colic junction. Further evaluation to determine the aetiology of the disease and classify it into the entity of inflammatory bowel disease is needed.

## ABSTRACT GI-28
## EVALUATION OF FECAL SHORT CHAIN FATTY ACID CONCENTRATIONS IN HEALTHY DOGS AND DOGS WITH CHRONIC DIARRHEA.

Y Minamoto, N Berghoff, VR Rangachari, JM Steiner,, JS Suchodolski. Gastrointestinal Laboratory, Texas A&M University, College Station, TX

Short-chain fatty acids (SCFA) are major end-products of bacterial carbohydrate fermentation in the gastrointestinal (GI) tract. They constitute an important energy source for intestinal epithelial cells and help maintain intestinal mucosal integrity. Several studies have shown decreased fecal SCFA concentrations in humans with inflammatory bowel disease. The role of SCFA in dogs with GI disease has not yet been studied extensively. Therefore, the aim of this study was to evaluate fecal concentrations of SCFA in healthy dogs and dogs with chronic diarrhea.

Fresh fecal samples were collected from 10 healthy pet dogs and 14 dogs with chronic diarrhea (>3 weeks duration). Fecal concentrations of SCFA (acetate, propionate, butyrate, and total SCFA) and branched chain fatty acids (BCFA; isobutyrate, isovalerate, valerate, and total BCFA) were measured using gas chromatography-mass spectrometry. The fecal SCFA concentrations were compared between both groups using a Student's *t*-test or Wilcoxon rank-sum test where appropriate. Significance was set at $p < 0.05$.

Fecal concentrations of acetate, propionate, and total SCFA were significantly decreased in dogs with chronic diarrhea when compared to the healthy dogs (Table 1). There were no significant differences in fecal concentrations of butyrate or any of the BCFA between groups.

In this study, dogs with chronic diarrhea had decreased fecal concentrations of total SCFA, acetate, and propionate. These results warrant further studies with larger populations of dogs and various GI diseases to further evaluate the role of SCFA in gastrointestinal health and disease in dogs.

**Table 1.** *Culicoides midges caught in tent traps on 3 farms with 2 horses per farm during 6 tests and in a black light trap.*

| | Total Culicoides | Culicoides blood-fed | Total *C.bsoletus* | *C.bsoletus* blood-fed |
|---|---|---|---|---|
| Horses with blanket | 402 | 130 (=32%) | 295 | 88 (=30%) |
| Horses without blanket | 464 | 260 (=56%) | 318 | 185 (=58%) |
| Black light trap | 1465 | 492 (=34%) | 1316 | 433 (=33%) |

## ABSTRACT GI-29
## THE EFFECT OF VARIOUS STORAGE CONDITIONS ON THE ABUNDANCE OF BACTERIAL GROUPS IN CANINE FECES.

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Quantitative polymerase chain reaction (qPCR) assays can be used to quantify specific bacterial groups in fecal samples of healthy dogs and dogs with clinical signs of gastrointestinal disease. The stability of fecal bacterial groups stored at various temperatures and for various time periods is of critical concern, because shifts in the fecal microbiota attributable to storage conditions may influence the interpretation of findings. Limited information is available about optimal storage conditions of fecal samples for evaluation of the microbiota. It is often recommended that samples be immediately frozen after collection. The aim of this study was to evaluate the effect of various storage temperatures and time periods on the abundance of selected fecal bacterial groups in dogs using qPCR.

Ten fresh fecal samples were collected from seven dogs. For each sample, 9 aliquots were prepared. One aliquot of each sample immediately underwent DNA extraction using a modified bead beating protocol (Zymo Fecal DNA extraction kit; Zymo-Research, Orange, CA). The remaining aliquots were stored at -80°C, -20°C, 4°C, and room temperature (RT). After 24 hours and after 7 days, one aliquot of each sample was extracted and analyzed. Previously established qPCR assays were utilized for the evaluation of abundance of total bacteria, gram positive bacteria (Firmicutes, *Faecalibacterium* spp., and *Clostridium perfringens*), and gram negative bacteria (Bacteroidetes). The bacterial abundance in all aliquots was compared using a repeated measures 2-Way ANOVA and a Bonferroni post-test with time and temperature as independent variables. A $p < 0.05$ was considered statistically significant.

Neither storage temperature nor storage time significantly influenced the abundance of total bacteria or gram positive bacteria. However, a lower abundance of Bacteroidetes was observed across all time points and storage conditions as compared to baseline abundance. Statistically significant differences were identified between the baseline samples (median = log 4.96 of DNA, range = log 4.02-5.43) and samples stored at -80°C for 24 hours (median = log 4.40, range = log 3.15-5.45 $p < 0.01$), those stored at -20°C for 24 hours (median = log 4.41, range = log 3.69-5.12; $p < 0.01$), those stored at -20°C for 7 days (median = log 4.49, range = log 3.00-5.35; $p < 0.05$), and those stored at RT for 7 days (median = log 4.46, range = log 3.67-5.43; $p < 0.05$).

In this study, neither temperature nor time had a significant influence on the abundance of Firmicutes, *Faecalibacterium* spp., or *C. perfringens*. However, a significantly lower abundance of

Bacteroidetes was observed within 24 hours of storage at -80°C and -20°C, and after storage at RT for 7 days. Because there was no significant change observed at 4°C for any of the bacterial groups analyzed, refrigeration may be recommended for short term storage of fecal samples. Future studies are needed to establish the influence of storage conditions on other clinically relevant bacterial groups.

**ABSTRACT GI-30**
**DAY-TO-DAY VARIATION OF FECAL MICROBIOTA IN DOGS OF DIFFERENT AGES.** JF Garcia-Mazcorro[1], JM Steiner[1], JS Suchodolski[1]. Gastrointestinal Laboratory, Texas A&M University, College Station, TX

Despite increasing evidence suggesting age-related differences in the composition and metabolic activity of the intestinal microbiota, the nature of this relationship remains unclear. Especially young dogs appear to be at an increased risk for gastrointestinal disease, which may or may not be related to changes of their intestinal microbiota. Therefore, the aim of this study was to investigate day-to-day variation of fecal microbiota in a population of healthy dogs of different ages.

A total of 22 dogs (median age: 6.2 years, range: 4 months - 12 years) were enrolled into this study. The dogs did not have any clinical signs of gastrointestinal disease and did not receive any medication within at least three months before enrolling into the study. Fecal samples were collected daily from all dogs during a period of five consecutive days. The abundance of total bacteria, *Enterococcus*, *Lactobacillus*, and *Streptococcus* spp. was estimated in feces using quantitative real-time PCR targeting the 16S rRNA gene using published oligonucleotides. The average, as well as day-to-day standard deviation and percentage coefficient of variation (%CV) were calculated using normalized 16S rRNA gene copies (16S rRNA gene copies from the bacterial groups divided by the 16S rRNA gene copies for total bacteria).

The day-to-day standard deviation for total bacteria ranged from 0.1 to 1.7, and from 0.2-0.5 for the three bacterial groups specifically analyzed (i.e., *Enterococcus*, *Lactobacillus*, and *Streptococcus* spp.). The day-to-day %CV for all bacteria ranged from 2-41%, and from 3-323% for the three bacterial groups analyzed separately. The bacterial group with the highest day-to-day variability was *Streptococcus* (median day-to-day %CV: 57%), followed by *Enterococcus* (median %CV: 34%), and *Lactobacillus* (median %CV: 6%). There was no significant linear relationship ($R^2 < 0.1$, $P > 0.1$) between age and either the average and day-to-day standard deviation or %CV for any of the bacterial groups analyzed, including total bacteria. Additionally, visual analysis of day-to-day variability plots did not reveal any obvious patterns associated with age. Instead, the pattern of day-to-day variability seemed to be unique in each dog and dependent on the specific bacterial group analyzed.

Our results suggest that short-term day-to-day variation of fecal microbiota is not affected by age in healthy dogs. Instead, this variation seems to be unique in each dog and dependent on the particular bacterial group analyzed. However, further studies are warranted to evaluate more intestinal microbial groups in a larger population of dogs to assess the relationship (if any) between aging and over time variability of the intestinal microbiota.

**ABSTRACT GI-31**
**PRESURGICAL EVALUATION OF RECTAL MASSES IN DOGS BY ENDOSCOPY AND ENDOSCOPIC ULTRASOUND: 28 CASES.** K Hayashi[1], H Okanishi[1], Y Sakamoto[1], Y Kagawa[2], K Asano[1], M Sakai[1], T Watari[1]. [1]Nihon University, Kanagawa, Japan, [2]North Lab, Hokkaido, Japan

Detailed evaluation of rectal masses is essential in selecting endoscopic treatment and minimal access surgery. However, because colorectal masses in dogs frequently occur in the distal rectum, visualization by abdominal ultrasound is difficult. The purpose of this study was to assess the usefulness of preoperative evaluation of rectal masses by endoscopy and endoscopic ultrasound (EUS).

This study targeted 28 cases in which rectal masses were assessed by endoscopy and EUS, prior to undergoing surgery. Abdominal ultrasound was performed in 10 cases, but was unable to provide detailed evaluation data. Rectal tumors in all cases could be evaluated in detail by EUS, and were classified by T stage (Tis-T2) or as submucosal tumor (SMT). Endoscopic histopathology showed good correlation with surgical histopathology in masses of Tis-T2 and accuracy was 92% ($\kappa = 0.85$). However, endoscopic and surgical pathology results did not correspond in SMT masses. Tis-T1 masses included 23 cases (inflammatory polyp: 15 cases; low-grade adenocarcinoma: 8 cases), T2 masses included 3 cases (high-grade adenocarcinoma: 2 cases; low-grade adenocarcinoma: 1 case) and SMT masses included 2 cases (leiomyoma: 1 case; rectal cyst: 1 case). EUS showed good correlation with surgical histopathology and accuracy was 86% ($\kappa = 0.72$).

Endoscopic histopathology was useful for evaluating of Tis-T2 masses, but was not useful in evaluating SMT masses. Diagnosis of SMT masses requires evaluation by both EUS and surgical histopathology. Although abdominal ultrasound was ineffective for evaluation of rectal masses, EUS was useful. Therefore, EUS may be applicable in the selection of surgical procedure and estimation of prognosis.

# SMALL ANIMAL - HEMATOLOGY

**ABSTRACT HM-1**
**THE OVERALL HAEMOSTASIS POTENTIAL; A NOVEL, COST EFFECTIVE, GLOBAL COAGULATION ASSAY FOR USE IN CANINE PLASMA.** A Dengate[1,2,3], MC Morel-Kopp[1], J Braddock[3], R Churcher[3], J Beatty[2], V Barrs[2], C Ward[1]. [1]Northern Blood Research Centre, Kolling Institute, University of Sydney, Australia, [2]Faculty of Veterinary Science, University of Sydney, Australia, [3]North Shore Veterinary Specialist Centre, Crows Nest, Australia

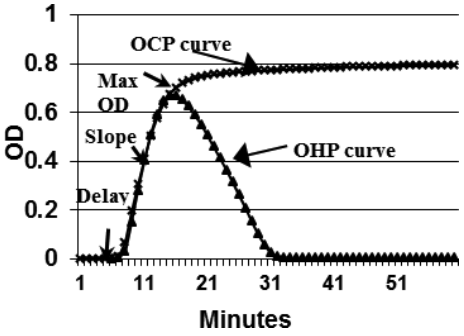
The overall haemostasis potential (OHP) is a novel global coagulation assay, measuring both speed and extent of coagulation as well as fibrinolysis (Figure 1). Other global haemostasis assays such as thromboelastography (TEG) and the calibrated automated thrombogram (CAT) are gaining popularity in veterinary medicine, though their application is limited by cost and the requirement for specialised equipment. The OHP measures optical density (OD), directly related to fibrin generation, with common absorbance spectrophotometry equipment. The purpose of this study was to optimise the OHP assay for use with canine platelet poor plasma, and correlate the outputs with those obtained by TEG and CAT.

Citrated plasma was collected from 60 clinically healthy dogs. The OHP assay and standard coagulation assays (prothrombin time [PT], activated partial thromboplastin time [APTT], fibrinogen [Fg], Factor VIII and von Willebrands factor [vWF]) were performed for each sample. CAT was performed on 23 samples, and TEG on seven.

Figure : Outputs generated by the OHP assay. The delay, maximum slope, and maximum optical density (OD Max) are recorded. The overall fibrinolytic potential is calculated by area under OCP – OHP/OCP x 100.

Modifications to the published methodology for the OHP assay were required, with less coagulation activator (thrombin) and more fibrinolysis activator (tPA) utilised in canine plasma. Increasing Factor VIII activity was associated with a shortened delay ($P = 0.043$). High fibrinogen levels and decreased PT were associated with increased OD Max, OCP, OHP and max slope ($P = < 0.05$). There was a strong correlation between the clot stability (G) and maximum amplitude (MA) using TEG, and the Delay, OCP, OD Max, OHP, and max slope obtained using the OHP assay ($P = < 0.05$). Comparing OHP assay with CAT, there was a positive correlation for Peak with the OHP curve ($P = 0.019$).

These results support the use of the OHP assay as an accessible, cost-effective alternative to TEG.




**ABSTRACT HM-2**

**MEASUREMENT OF THROMBIN GENERATION IN DOGS WITH DIFFERENT DISEASES.** R Mischke[1], C Witten[1], A Tiede[2]. [1]Small Animal Clinic, University of Veterinary Medicine Hannover, Hannover, Germany, [2]*Department of Haematology*, Haemostasis. Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

The aim of the present study was to establish reference values for the in vitro thrombin generation (TG) assay and to assess possible changes in dogs suffering from different diseases which may be associated with hypo- and/or hypercoagulability.

Dogs with hemophilia A (5), hemophilia B (3), hyperadrenocorticism (11), inflammatory diseases (11) and neoplastic diseases (lymphoma [7], systemic histiocytic sarcoma [6], acute lymphoblastic leukaemia [5]) were included. 62 healthy dogs served as a control. TG was measured by use of the Calibrated automated Thrombinography (CAT) method which revealed the following parameters: lag time, peak, endogenous thrombin potential (ETP), time to peak and reaction time. Measurements were carried out with three different TF concentrations (1, 5 and 20 pM) using frozen platelet poor citrated plasma as sample material.

Dogs with hemophilia showed a significantly lower peak and prolonged lag time, time to peak and reaction time at low TF concentrations when compared to healthy dogs,. Dogs with hemophilia B showed evidence of a lower mean peak and ETP at a TF concentration of 1 pM. There was a trend towards more severe changes with decreasing residual factor activity. Dogs with hyperadrenocorticism and inflammatory processes had significantly higher ETPs at all TF concentrations in comparison to healthy dogs, whereas ETP in neoplastic diseases was mainly unchanged. Dogs with histiocytic sarcoma had increased ETP values at 1 pM TF.

TG, particularly when performed with 1 pM TF, seems to be a valuable additional tool to verify hypo- and hypercoagulability in dogs and may be a helpful indicator of severity of thrombophilia in hypercoagulable conditions and a predictor of the severity of hemorrhagic diathesis in dogs with hemophilia.

**ABSTRACT HM-3**

***IN VITRO* EVALUATION OF THE EFFECT OF RIVAROXABAN ON COAGULATION PARAMETERS IN HEALTHY DOGS.** B Conversy, M-C Blais, C Gara-Boivin, JRE del Castillo, L Carioto, M Dunn. Faculté de Médecine Vétérinaire, Université de Montréal, Montreal, Canada

Rivaroxaban is a novel, direct factor Xa inhibitor used in people for the prevention and treatment of thromboembolic disorders at a fixed-dose regime, without the need for laboratory monitoring. Its *per os* administration makes it an attractive anticoagulant for dogs. The purpose of this study was to evaluate the *in vitro* effects of rivaroxaban on canine coagulation parameters.

Aliquots of pooled citrated platelet poor plasma obtained from 20 healthy adult beagles were fortified *in vitro* with DMSO solutions of rivaroxaban (98% purity) to obtain 19 final concentrations ranging from 0 (solvent) to 1000 mg/L of drug. Samples were immediately submitted for the following coagulation assays: prothrombin time (PT), partial thromboplastin time (PTT), tissue factor induced thrombin generation and anti-factor Xa activity. Concentration-effect data were analyzed with ADAPT v.5, using sigmoid stimulatory or inhibitory Emax regression models.

Rivaroxaban caused a concentration-dependent prolongation of all coagulation parameters. The half maximal inhibitory concentration (IC50) for the propagation phase (rate index) of thrombin generation was 0.03 mg/L. The anti-Xa activity recommended for thromboprophylaxis (0.5-1 U/mL) was reached at rivaroxaban concentrations between 0.04 and 0.11 mg/L. At these concentrations, PT and PTT remained within the reference range. Two-fold prolongation from baseline of PT and PTT were achieved with concentrations of 1.24 and 1.45 mg/L, respectively. Thrombin generation was completely suppressed with concentrations $\geq$ 0.8 mg/L.

*In vitro* concentration-effect relationships of rivaroxaban on canine plasma coagulation parameters were documented. Thrombin generation and anti-Xa activity appear more sensitive in detecting rivaroxaban's anticoagulant effect than PT and PTT.

**ABSTRACT HM-4**

**ASSESSMENT OF HYPERCOAGULABILITY IN CANINE PITUITARY-DEPENDENT HYPERADRENOCORTICISM.** F Park, A Abrams-Ogg, D Wood, D Allen, S Blois. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

Dogs with pituitary-dependent hyperadrenocorticism (PDH) are at increased risk of thromboembolic disease. Thromboelastography (TEG®) provides a global assessment of coagulation, and is more sensitive than conventional coagulation tests for detecting hypercoagulability. TEG® with Platelet Mapping™ allows assessment of maximal amplitude (MA) in response to the agonists arachidonic acid ($MA_{AA}$) and adenosine diphosphate ($MA_{ADP}$).

The purpose of this prospective clinical study was to evaluate for, and quantify, hypercoagulability in dogs with PDH. Citrated kaolin TEG® with Platelet Mapping™ was performed in 19 dogs with PDH pre-treatment, in 16 of these dogs during treatment with trilostane, and in 40 healthy control dogs. For citrated kaolin TEG®, dogs with PDH had decreased k-time ($p = 0.002$), increased angle ($p = 0.007$) and increased MA ($MA_{thrombin}$) ($p < 0.0001$) compared to controls. The $MA_{thrombin}$ of dogs treated with trilostane decreased significantly after 3 months ($p = 0.02$) and 6 months ($p = 0.04$), but was still significantly higher than controls. $MA_{AA}$ was significantly higher in dogs with PDH ($p = 0.02$) but did not change significantly following treatment. $MA_{ADP}$ was not significantly different between PDH dogs and controls.

In conclusion, dogs with PDH have evidence of increased clot strength and rate of clot formation. Clot strength decreases significantly during trilostane treatment, but does not normalize. Results also suggest that dogs with PDH may have higher platelet reactivity to arachidonic acid.

**ABSTRACT HM-5**

**DELAYED FIBRINOLYSIS IN DOGS WITH NATURALLY OCCURRING PITUITARY DEPENDANT HYPERADRENOCORTICISM.** A Dengate[1,2,3], MC Morel-Kopp[1], J Braddock[3], R Churcher[3], J Beatty[2], V Barrs[2], C Ward[1]. [1]Northern Blood Research Centre, Kolling Institute, University of Sydney, Australia, [2]Faculty of Veterinary Science, University of Sydney,

Australia, [3]North Shore Veterinary Specialist Centre, Crows Nest, Australia

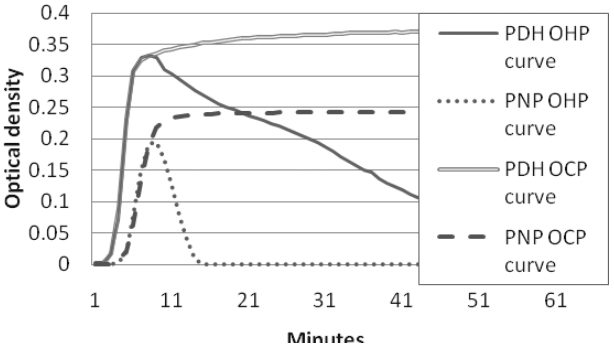
Canine hyperadrenocorticism has been associated with hypercoagulability due to increases in circulating procoagulant factors II, V, VII, IX, X, XII, and fibrinogen, and decreased anticoagulant antithrombin (AT). In human hyperadrenocorticism, increased plasminogen activator inhibitor (PAI-1) also contributes to impaired fibrinolysis. The purpose of this study was to identify hypercoagulability in dogs with pituitary-dependent hyperadrenocorticism (PDH), using a novel global coagulation assay, the overall haemostasis potential (OHP).

Citrated plasma samples were collected from 11 client owned dogs with PDH, and 11 age-matched, clinically healthy dogs. The OHP assay was run on each sample, recording optical density (related to fibrin generation and lysis) over 1 hour (Figure 1).

The mean area under the OHP curve was significantly increased in the PDH group (8.9) compared with the normal group (2.2), P = 0.038 (student's *t*-test). The fibrinolytic potential (area under OCP – OHP/OCP x 100) was significantly reduced in PDH group (51.45%) compared with the normal group (84.68%), P = 0.017.

These results support the presence of a hypercoagulable state associated with canine PDH, and that delayed fibrinolysis contributes to this state.

**Figure** : Overall haemostasis potential (OHP) and overall coagulation potential (OCP) curves for canine PDH and pooled normal canine plasma (PNP). The OHP curve shows both coagulation and fibrinolysis, the OCP coagulation only.




**ABSTRACT HM-6**

**SYRINGE AND AGGREGATE FILTER ADMINISTRATION DOES NOT AFFECT SURVIVAL OF TRANSFUSED AUTOLOGOUS FELINE RED BLOOD CELLS.** B Heikes, C Ruaux. Oregon State University College of Veterinary Medicine, Corvallis, OR

The use of mechanical administration systems, such as fluid pumps and syringe + aggregate filters, are reportedly associated with a risk of early loss of transfused autologous red blood cells (RBC's) in the dog. Of these two techniques, syringe + aggregate filter is more commonly used in the cat. As cat RBC's have differing flexibility and size than canine RBC's, the aim of this study was to determine the effect of syringe + aggregate filter administration on RBC's from domestic cats. Our hypotheses were that administration of autologous feline RBC's through a syringe + aggregate filter would decrease the probability of short-term survival of the transfused cells, and reduce the circulating half-life of transfused cells, when compared to control RBC's administered via gravity flow.

This was a prospective, blinded study. Six apparently healthy cats (minimum 4.5 kg bodyweight) belonging to staff and students of the Oregon State College of Veterinary Medicine were enrolled. Whole blood (40 ml/cat) was collected into 2x25ml syringes containing CPDA-1 anticoagulant. Each aliquot of blood was centrifuged, plasma was harvested and stored, and then RBC's were washed twice in PBS before suspension in 40ml of PBS + 1%Dextrose. Washed cells were biotinylated at one of two biotin densities. After biotinylation, cells were washed twice in PBS, packed by centrifugation, and then suspended in autologous plasma before transfer to a labeled sterile syringe or 50ml blood bag. Each cat was then transfused with both aliquots of autologous labeled blood, delivered at a rate of 2 ml/kg/hr using either syringe pump + aggregate filter (AS-50 Syringe Pump, Baxter & Hemo-Nate® Filter, Utah Medical Products) or gravity flow via a standard blood-giving set. After transfusion, 1ml blood samples were collected into EDTA at 2-hour intervals for 12 hours. Subsequent 1 ml samples were obtained weekly for 7 weeks. Five µL aliquots of each whole blood sample were washed in PBS, incubated with streptavidin-phycoerythrin, and then analyzed by flow cytometry. The proportion of transfused cells remaining in each of the two populations was measured. Quantitative changes in the two populations over time were assessed by two-way repeated measures ANOVA.

Biotinylated RBC's were readily detected in all samples from all cats over the 7-week sampling period. One cat showed early loss of RBC's delivered via gravity flow. There was a significant decrease in both populations of labeled cells over the 7-week period ($p < 0.01$), as expected. There was no significant difference in probability that the RBC's would survive 12 hours immediately following transfusion, and no significant difference in survival between the two groups over 7 weeks. The average half-life of all labeled cells was approximately 42 days.

We conclude that the use of a syringe + aggregate filter method to transfuse cats with autologous RBC's is not associated with decreased short-term RBC survival or decreased RBC half-life.

**ABSTRACT HM-7**

**LEUKOREDUCTION OF FELINE WHOLE BLOOD USING A NEONATAL LEUKOCYTE REDUCTION FILTER: A PILOT EVALUATION.** J Schavone[1], E Rozanski[1], J Schaeffer[2], L Monhait[1], CR Sharp[1]. [1]Section of Critical Care Tufts Cummings School of Veterinary Medicine, North Grafton, MA, [2]Pall Corporation, Port Washington, NY

Leukocytes (WBC) present in stored blood are associated with a variety of potentially deleterious pro-inflammatory and immune responses. Pre-storage leukocyte reduction (LR) has been advised to reduce the WBCs in stored blood. While LR has been described in human and canine transfusion medicine, due to sizing of available filters, LR has not been previously evaluated in cats. The purpose of this pilot study was to evaluate the efficacy of a human neonatal LR filter in leukoreducing feline fresh whole blood (FWB) using different techniques.

Sixty milliliters of FWB was obtained from 8 healthy donor cats using standard techniques. LR was achieved using an in-line LR filter. 3 different techniques were assessed. Group 1) Warm (room temperature) FWB filtered immediately via slow active push (n = 2), Group 2) Warm FWB filtered immediately via gravity (n = 3), and Group 3) FWB, chilled at 4°C for 60 min, filtered via gravity. Efficacy of filtration was assessed by the pre- to post-filtration decrease in leukocyte count (WBCC) using a Cell-Dyn Hematology Analyzer, with a level of detection of 500 cell/ul. LR of chilled blood filtered by gravity was significantly more effective (100% LR, p = 0.001) than warm FWB filtered actively (35%) or by gravity (59%).

Inadequate LR was achieved in room temperature blood with active or gravity filtration.

We conclude that chilled FWB can be successfully LR pre-storage with gravity flow through this in-line neonatal filter. The main limitation of the technique is loss of blood volume, since the residual filter volume is 8mL. Investigation of the potential therapeutic benefit of using LR blood in cats is warranted.

**ABSTRACT HM-8**

**UTILITY OF SERIAL ANTI-XA MONITORING IN DOGS RECEIVING DALTEPARIN.** AM Lynch, AM DeLaforcade, CR Sharp. Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, Massachusetts

Dalteparin is commonly employed as an anticoagulant in critically ill dogs. The aim of this study was to describe the clinical course of hospitalized dogs receiving dalteparin serially monitored with a chromogenic anti-Xa assay.

The medical records of 24 dogs were retrospectively reviewed. Underlying diseases included immune mediated hemolytic anemia, sepsis, neoplasia and protein losing diseases. Five dogs had confirmed thromboembolism prior to anticoagulation. Nineteen dogs were hypercoagulable based on thromboelastography. The median dalteparin starting dose was 150 IU/kg SQ. Twice daily dosing was chosen for 14/24 dogs, three times daily for 9/24 dogs and four times daily for the remaining dog. Anti-Xa assays were performed 4 hours post-administration of dalteparin in 20 dogs and at trough for 4 dogs. From the initial dalteparin dose 7 dogs achieved target anti-Xa levels (0.5-1.0 units/mL), 10 dogs achieved levels above the target range and 6 dogs were below range. Individual dose adjustment was made in 19 dogs. Ultimately 20/24 dogs either achieved or exceeded the target anti-Xa range. Minor bleeding complications (subcutaneous hemorrhage at the injection site) were noted in 2 dogs. Nineteen dogs survived to discharge; 5 were euthanized due to clinical deterioration. Three dogs had confirmed thromboemboli at necropsy.

Serial anti-Xa assays were useful to guide individual dose adjustment of dalteparin in dogs in this study. Further studies are warranted to understand the impact of individual dose adjustment on outcome and cost effectiveness of dalteparin treatment in critically ill dogs at risk of thromboembolism.

## ABSTRACT HM-9

**PLATELET FUNCTION IN DOGS WITH INFLAMMATORY DISEASES.** R Mischke, M Abid, K Kalbantner. Small Animal Clinic, University of Veterinary Medicine Hannover, Germany

The aim of this study was to examine the influence of inflammatory diseases on primary hemostasis in dogs.

Six different parameters were used for testing platelet function in 25 dogs with bacterial or protozoal infections: capillary bleeding time, automatic platelet function analysis (PFA 100, collagen/ adenosine diphosphate [COL/ADP] and collagen/epinephrine cartridges), turbidimetric platelet aggregation, impedance aggregometry using a multiple electrode aggregometer, platelet count and hematocrit. Results of the 25 dogs with inflammatory diseases were compared to the control group and additionally classified into two subgroups based on criteria of systemic inflammatory response syndrome (SIRS) (groups "SIRS" and "Non-SIRS").

The following findings were observed in dogs with inflammatory diseases when compared to the control group: a significantly prolonged closure time of the automatic platelet function analyser measured with both cartridges (e.g. COL/ADP: 83 s [55–301 s] vs. 65 s [47–99 s]; median [minimum–maximum]; $P < 0.0001$), a significant decrease in maximal aggregation of the ADP-induced turbidimetric aggregometry (e.g. 40 µmol/L: 45.2 ± 26.8 % vs. 67.3 ± 21.8 %; mean ± SD; P = 0.003) and collagen, a significant increase of impedance aggregometry induced with 5 µg/mL collagen, and significant suppression of arachidonic acid-induced impedance aggregometry. A higher collagen-induced impedance aggregation which was detected in dogs suffering from "SIRS" was the only significant differences between subgroups "SIRS" and "Non-SIRS".

Although individual tests indicate enhanced platelet aggregation, results of in vitro tests in the present study mainly reveal a normal or minimally to moderately reduced functionality of platelets. The reduced response to aggregation agonists in vitro may partly attributed to the fact that platelets are already activated under inflammatory conditions.

## ABSTRACT HM-10

**15-GAUGE CORE BONE MARROW BIOPSY IN SMALL DOGS AND CATS WITH HEMATOLOGIC DISORDERS – 16 CASES.** A Abrams-Ogg, A Defarges, D Bienzle. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

The diameter of 11-13ga Jamshidi-type needles makes bone marrow (BM) core biopsy difficult in small dogs and cats. We previously reported the feasibility of 15ga BM biopsy in normal small dogs (Vet Clin Pathol, in press) and cats (J Vet Intern Med 2011;25:703) using EZ-IO® (Vidacare) needles. In this study, 15-25 mm EZ-IO needles were used by 5 operators with variable procedural experience to obtain humeral head BM biopsies from anesthetized small dogs and cats diagnosed with non-regenerative anemia, neutropenia or pancytopenia between June 2009 and October 2011. The EZ-IO cannulae were advanced and retracted manually without a power driver. Specimens were placed into Davidson's fixative for 20 min of rapid fixation, and then transferred to formalin. Sternal or humeral BM aspirates were also obtained. Animals were treated post-biopsy with opioid or NSAID analgesics and assessed for pain and lameness. The tips of the cannulae, which are crown-shaped, were inspected for damage. Biopsies were reviewed by a pathologist. Biopsy length on the slide was measured, and biopsy quality was scored from 0 (no hematopoietic tissue) to 5 ($\geq 5$ intertrabecular spaces free of artifact). Results [Mean ± SD (range)] were:

Quality and length were not different between dogs and cats (P = 0.3-0.4). Biopsy quality and length were significantly correlated (rho = 0.74, P = 0.002). There were no correlations between animal weight and biopsy quality or length. Six biopsies had quality scores of 4-5; of these, 5 were obtained on the first attempt. For 4 biopsies, the cannulae tip were pinched closed; these biopsies were of higher quality (4 – 5) and length (9 mm). Although quality scores of 1-2 were given for 10 biopsies, even these encompassed sufficient hematopoietic tissue to support a diagnosis in conjunction with hemograms and BM cytology. Diagnoses in dogs included myelofibrosis (1), erythroid hypocellularity (2), myeloid hypocellularity (1), trilineage hypocellularity (1), myeloid hyperplasia (1), myeloid leukemia (1) and myelodysplasia (1). Diagnoses in cats included myelofibrosis (1), erythroid hypocellularity (3), bi- or trilineage hypocellularity (2), erythroleukemia (1), and lymphoid leukemia (1). For 5 dogs and 5 cats, BM core biopsy was needed for diagnosis; for 3 dogs and 3 cats, biopsy confirmed the cytology diagnosis. In conclusion, 15ga core BM biopsy is a well-tolerated procedure that may facilitate diagnosing hematopoietic disorders in small dogs and cats. Intentional damage (pinching) of the cannula tip on the first pass through the BM may serve to facilitate specimen retention and maximize quality.

**Abstract HM-10: Table**

| Patients (n) | Age (yr) | Weight (kg) | 24h pain or lameness | Biopsies (n) | Quality (1 - 5) | Length (mm) |
|---|---|---|---|---|---|---|
| 8 dogs | 7.9 ± 1.8 (5.8 – 11.0) | 6.0 ± 2.3 (2.0 – 9.4) | None | 10 | 2.6 ± 1.9 (0 – 5) | 6.2 ± 3.0 (0 – 11) |
| 8 cats | 5.1 ± 4.4 (0.8 – 12.2) | 4.6 ± 1.1 (2.6 – 5.9) | None | 9 | 1.8 ± 1.5 (0 – 4) | 5.2 ± 2.0 (2 – 9) |

## ABSTRACT HM-11

**SERUM BIOMARKERS INDICATE HEMOLYSIS AND AN INFLAMMATORY RESPONSE TO TRANSFUSION OF AUTOLOGOUS STORED ERYTHROCYTE CONCENTRATES.** SA Smith[1], M McMichael[2], JM Herring[2], A Galligan[2], R Corsi[2], T Ngwenyama[2], AN Beloshapka[3], P Deng[3], KS Swanson[2,3]. [1]College of Medicine, [2]College of Veterinary Medicine, or [3]Department of Animal Sciences, University of Illinois, Urbana, IL

We previously reported that transfusion to normal dogs of stored (21 days) autologous erythrocyte concentrates (pRBCs) caused an inflammatory response as indicated by leucocytosis and elevations in the acute phase reactants. Furthermore, pRBCs

stored for 35 days elicited a degenerative left shift followed by a regenerative left shift. We hypothesized that transfusion of fresh pRBCs would elicit less profound increases in serum biomarkers of inflammation than would stored pRBCs.

A whole blood unit was collected from healthy dogs (n = 10) for pRBCs on day 0, then again on day 32. On day 35 dogs received an autologous transfusion of pRBCs stored for either 35 days (S, n = 5) or 3 days (F, n = 5). Serum free hemoglobin (Hgb), CRP, interleukin-8 (IL-8), tumor necrosis factor α (TNFα) were evaluated on serum samples collected at 0 (pre) and 5,9,24,48, and 72 hours after transfusion.

Fresh pRBCs did not elicit any change in serum Hgb, or CRP. IL-8 and TNFα both increased approximately 2 fold, with peaks at 5 hours and 24 hours respectively. Stored pRBCs elicited a 60 fold increase in free serum Hgb (mean 0.21, SD 0.06 g/dL) with a peak at 9 hours. CRP also profoundly increased up to 90 fold with a peak at 24 hours (mean 91, SD 33 μg/mL). IL-8 and TNFα both increased approximately 2 fold, with peaks at 5 hours and 9 hours respectively.

More significant hemolysis occurs with transfusion of stored erythrocytes. Transfusion of autologous stored pRBCs elicits a markedly greater inflammatory response than fresh pRBCs.

## ABSTRACT HM-12

## ASSESSMENT OF PLATELET ACTIVATION IN DOGS ADMITTED TO THE INTENSIVE CARE UNIT (ICU) BY USE OF FLOW CYTOMETRY AND THROMBOELASTOGRAPHY.

SB Majoy[1], AM de Laforcade[1], M Barnard[2], A Hoffman[1], SP Shaw[1]. [1]Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, [2]University of Massachusetts Medical School, Worcester, MA

Thromboembolic disease is a potentially life-threatening complication in critically ill dogs and may be related to excessive platelet activation. We hypothesized that dogs admitted to the ICU would exhibit increased platelet activation as measured by flow cytometry and thromboelastography compared to normal dogs and that there would be a correlation between the degree of platelet activation and severity of illness.

81 dogs hospitalized in the ICU and 24 normal control dogs were enrolled. Blood was collected into 3.2% sodium citrate tubes by jugular venipuncture and rested 30 minutes prior to analysis. Platelet activation was determined by flow cytometry using monoclonal mouse anti-human CD61 and CD62 antibodies in resting and ADP-activated samples. Thromboelastography was performed using kaolin as an activator. SPI2 and APPLE scoring systems were used to estimate severity of illness.

The degree of platelet activation at rest was not significantly different between control dogs and sick dogs as measured by percent positive platelets (%P; 1.34 vs 0.79, $p = 0.23$) or mean fluorescence intensity (MFI; 2314 vs. 798, $p = 0.72$). Compared to control dogs, sick dogs had significantly increased platelet activation in response to 2 uM ADP (23.5 vs. 2.9 %P, 2425 vs. 1235 MFI), 6 uM ADP (36.6 vs. 13.3%P, 2722 vs. 1783 MFI), and 10 uM ADP (3562 vs. 1306 MFI; $p < 0.05$). Sick dogs had a significantly increased MA (61.9 vs. 54.5), alpha (62.5 vs. 56.2), and G (9723 vs. 5802), and a significantly decreased K time (2.3 vs. 2.7) compared to controls. MA was negatively correlated with %P and MFI in sick dogs. No significant association was found between SPI2, APPLE, and degree of platelet activation.

There is evidence of increased platelet activation in dogs hospitalized in the ICU, but the SPI2 and APPLE scoring systems are not predictive of the degree of activation.

## ABSTRACT HM-13

## EVALUATION OF THROMBOELASTOGRAPHY TO PREDICT CLINICAL BLEEDING IN THROMBOCYTOPENIC DOGS AFTER TOTAL BODY IRRADIATION AND HEMATOPOIETIC CELL TRANSPLANTATION.

MC Bucknoff, R Hanel, S Marks, A Motsinger-Reif, S Suter. North Carolina State University College of Veterinary Medicine, Raleigh, NC

The objective of this prospective observational study was to correlate results of clinical hemostatic assays to bleeding events in a cohort of thrombocytopenic dogs. Ten client-owned dogs with lymphoma undergoing total body irradiation and autologous hematopoietic cell transplantation were sequentially enrolled. Bleeding events were separated into clinical bleeding (CB), including melena, hematochezia, hematemesis, epistaxis and hematuria, and minor surface bleeding (MSB), defined as new petechia or ecchymoses. Physical examinations, complete blood counts, and serum chemistries were performed daily on all patients and used to trigger data collection, which included kaolin activated thromboelastography [TEG, including reaction time, clot formation time, angle, maximal amplitude (MA), and global clot strength], buccal mucosal bleeding time, prothrombin time, activated partial prothrombin time, d-dimers, and fibrinogen. Data was captured at hospital admission, when platelet counts dropped below 30,000/μl, below 10,000/μl, or rebounded above 10,000/μl, and within 12 hours of a CB event.

Association analyses were performed for each assay variable with Cox Proportional Hazards analysis, followed by a Bonferroni correction. Groups compared included: 1) No bleeding or MSB compared to CB; 2) MSB compared to no bleeding; 3) CB compared to MSB; 4) CB compared to no bleeding. There was a statistically significant association of the TEG value MA for groups 1, 3, and 4. No significant association was found for any other analyzed variable. In conclusion, MA was the only coagulation parameter that was associated with signs of clinical bleeding.

## ABSTRACT HM-14

## IMMUNE-MEDIATED HEMOLYTIC ANEMIA IN CATS WITH PANCREATITIS.

A Zoia. "San Marco" Veterinary Clinic, Padua, Italy

Pancreatitis has been demonstrated in humans and experimentally in rats as a complication of acute hemolysis. Following the identification of a cat with acute hemolysis due to IMHA and concurrent pancreatitis, the aim of this study was to retrospectively investigate if these two diseases are associated in cats.

For this retrospective, case-control study, the author's residency case log of all cases seen at Glasgow University Small Animal Hospital between July 2004 and December 2007 (consisting of 157 client owned feline patients and 674 canine patients), was searched for cats diagnosed with pancreatitis. These cats were included in group 1. A clinical diagnosis of pancreatitis was made on a combination of: clinical signs, measurement of serum feline pancreatic lipase (fPLI) or trypsin-like immunoreactivity (fTLI), and abdominal ultrasonography. A control population sick cats without pancreatitis (group 2) was created from the same database. For each cat in group 1, three sick cats were included in group 2. These cats were randomly selected after age-, sex-(including neutered status) and breed-matching to the cats with pancreatitis. IMHA was diagnosed as a hematocrit $\leq$ 15% and the presence of a positive Coombs' test. The prevalence of IMHA between the two groups of cats was calculated and the difference in prevalence was statistically evaluated with the Fisher exact test. The level of significance was set as $p < 0.05$.

Of the 157 cats included in the database, 9 were diagnosed at presentation with pancreatitis and three of these cats (33%) had a concurrent diagnosis of IMHA. Only 1 (3.4%) of the 27 cats in group 2 (sick cats without pancreatitis) was diagnosed with IMHA. The prevalence of IMHA was significantly higher in group 1 compared with groups 2 ($p = 0.04$).

Cats with pancreatitis have an higher prevalence of IMHA compared to other sick cats. The hemolysis occurring during IMHA may therefore be the etiological cause of this disorder in these cats, as previously reported in humans and rats.

## ABSTRACT HM-15

## OSMOTIC FRAGILITY AND FLOW CYTOMETRIC DETERMINATION OF LIPID PEROXIDATION IN FELINE ERYTHROCYTES.

R Timmons, BF Goodrich, M Gillette, CB Webb. Colorado State University, Fort Collins, CO

Vitamin E (vit E) antioxidant properties include reducing lipid peroxidation. Antioxidant capacity in units of vit E is significantly decreased in cats with chronic kidney disease (CKD) com-

pared to controls (56 ± 21 vs. 81 ± 13, $p < 0.005$). With the goal of allowing further assessment of the role of oxidative stress and vit E in the anemia of CKD, we developed functional assays of feline RBC health.

Osmotic fragility was measured in cats with a range of packed cell volumes (PCV) using a standardized protocol. The variance, effect of sample dilution, and effect of storage time was determined. An r-squared of 0.45 described the fit between PCV and the NaCl concentration that produced 50% hemolysis ($n = 15$).

A protocol for the flow cytometric determination of lipid peroxidation in feline RBCs was developed using the fluorescence attenuation of the DHPE fluorophore, which is inversely proportional to membrane lipid peroxidation. DHPE treated samples ($n = 8$) run in triplicate gave a mean fluorescence of 1129 units, with a mean standard deviation of 41.0 units (4%). DHPE treated samples ($n = 10$) run before and after incubation with cumene hydroperoxide to stimulate lipid peroxidation showed an average decrease in fluorescence of 18% (+/- 12%) after stimulation. An r-squared of 0.75 described the fit between PCV and DHPE fluorescence ($n = 10$).

These assays can be used to determine the correlation between RBC membrane health and vit E antioxidant capacity, as well as the effect of vit E supplementation, in anemic cats with CKD.

**ABSTRACT HM-16**
**POWER-DRIVER ASSISTED 11-GAUGE HUMERAL CORE BONE MARROW BIOPSY IN LARGE BREED DOGS WITH HEMATOLOGIC DISORDERS – 6 CASES.** A Abrams-Ogg, A Defarges, D Bienzle. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

Bone marrow (BM) core biopsy is often performed using 11-13ga Jamshidi-type tapered needles and manual power. Anecdotally, some operators noted poor specimen retention. A newer biopsy system (OnControl, Vidacare) consists of a trocar-tipped stylet; a trephine with an internal marrow capture thread; and a power driver. In this study, the OnControl system was used by 6 different operators with variable procedural experience to obtain humeral head BM biopsies from 6 anesthetized large-breed dogs with hematologic disorders diagnosed between Nov 2010 and 2011. Specimens were placed in formalin, and concurrent sternal or humeral BM aspirates were obtained. Biopsy length on the slide was measured, and biopsies were reviewed and quality scored from 0 (no hematopoietic tissue) to 5 ($\geq 5$ intertrabecular spaces free of artifact) by a pathologist. Dogs were treated with opioid or NSAID analgesics post-biopsy. Chronologic results were:

For cases 2 and 5 there was difficulty orienting the biopsy needle and advancing through the cortex, and there was evidence of mild necrosis at the proximal end of these biopsies. Once the cortex was penetrated and the stylet removed, all operators reported that the trephine was very easy to advance and retract, and this phase of the biopsy was completed in $< 1$ min. All biopsies captured specimens on the first attempt. In the last dog the biopsy was repeated as the specimen was grossly of lower quality than in the previous dogs. One biopsy had a quality score of 1; this was due to fragmentation, but the specimen was nevertheless adequate for diagnosis. No dog showed signs of lameness attributable to the procedure. In conclusion, the OnControl system reliably captured humeral BM biopsies from large dogs with various hematologic disorders. In 3 cases BM core biopsy was needed for diagnosis; in 3 cases results were confirmatory.

**Abstract HM-16: Table**

| Dog # - Breed | Wt (kg) | Age (yr) | Hematologic disorder | Length (cm) | Quality (0-5) | Histologic diagnosis | Aspirate diagnosis |
|---|---|---|---|---|---|---|---|
| 1-Giant Schnauzer | 50 | 8 | Nonregenera- tive anemia | 2.7 | 5 | Myelo-fibrosis | Non-diagnostic |
| 2-Standard Poodle | 29 | 8 | Pancytopenia | 2.6 | 5 | Myelo-dysplasia | Myelo-dysplasia |
| 3-Lurcher | 28 | 3 | Nonregenera- tive anemia | 3.5 | 5 | Myelo-fibrosis | Non-diagnostic |
| 4-Cane Corso X | 51 | 3 | Pancytopenia | 2.4 | 5 | Myelo-suppression | Myelo-suppression |
| 5-Mixed Breed | 33 | 12 | Thrombo-cytopenia | 1.2 | 1 | MGK hyperplasia | MGK hypoplasia |
| 6-Labrador Retriever | 28 | 7 | Monoclonal gammopathy | 1.4 | 3 | Multiple myeloma | Multiple myeloma |
| | | | | 1.0 | 5 | | |

**ABSTRACT HM-17**
**EVALUATION OF A HUMAN ELISA KIT FOR THE MEASUREMENT OF PLASMA ADAMTS13 ACTIVITY IN DOGS.** H Maruyama, T Ohtake, M Kaneko, R Kano, Y Yamaya, T Watari, H Kamata. Nihon University, School of Veterinary Medicine, Kanagawa, Japan

A disintegrin-like and metalloprotease with thrombospondin type 1 repeat motifs 13 (ADAMTS13) is a von Willebrand factor (VWF)-cleaving protease. Deficiency in ADAMTS13 activity is known to cause thrombotic diseases in humans. The minimal substrate for ADAMTS13 in humans is VWF73, which contains 73 amino acid residues in the A2 domain of VWF. The aims of this study were to clarify whether canine ADAMTS13 has the ability to cleave human VWF73, and to test a human ADAMTS13 ELISA kit containing human VWF73 for the measurement of plasma ADAMTS13 activity in dogs.

Human VWF73 fused with a GST-tag and a His-tag was expressed in *Escherichia coli* and was purified (GST-VWF73-His). The recombinant canine ADAMTS13 (rcADAMTS13) was expressed in Hela cells, and the concentrated supernatant was used. The GST-VWF73-His was incubated with serially diluted rcADAMTS13, canine pooled plasma, or human pooled plasma. The cleaved products were detected by Western blotting with anti-GST antibody. In all samples, the signals of the cleaved products increased with increasing ADAMTS13 activity.

An ADAMTS13 ELISA kit with human VWF73 (KAINOS Lab., Tokyo) was used to measure canine plasma ADAMTS13 activity. Thirteen citrated plasma samples were obtained from 7 healthy dogs and 6 dogs with bacteremia. The ADAMTS13 activity was significantly lower in dogs with bacteremia than in healthy dogs ($p = 0.019$).

These results revealed that human VWF73 is cleaved efficiently by canine ADAMTS13 and the ELISA kit with human VWF73 can be useful for measuring plasma ADAMTS13 activity in dogs for the study of thrombotic disorders.

# SMALL ANIMAL – HEPATOLOGY

**ABSTRACT HP-1**
**LIVER HISTOLOGICAL LESIONS IN 43 SCOTTISH TERRIERS (ST) WITH HYPERACTIVITY OF ALKALINE PHOSPATESES (AP).** M Chevallier, S Guerret, A Pagnon, P Lecoindre, C Peyron, France

ST increased AP level was associated with development of a vacuolar hepatopathy (VH) as shown in previous study on 13 dogs (Lecoindre 2009). To assess liver histological lesions in a 43ST with increased AP level (mean: 10N). 55 liver samples obtained from 43 ST (16 wedge biopsies, 39 guided fine-needle biopsies), were analyzed including histological techniques: HES, Trichrome, Picrosirius/PERLS stains, semi-quantitative assessment of vacuolar hepatocytes using Center grading (G1 to G4)

(JAVMA 2006) necrotic-inflammatory activity (A0 to A3) and fibrosis (F0 to F4) according to METAVIR Score (1996). Cancer or architectural changes were recorded. Ultrastructural study (TEM) for two representative animals and α-smooth muscle actin antibody (αSMA) immunohistochemistry (IHC) analysis for cells involved in fibrogenesis were undertaken.

Mean age was 8 years (2-13) and ratio M/F (21/22). Lesions distribution was: vacuolar changes (G1 = 10, G2 = 15, G3 = 9, G4 = 9), fibrosis (F0 = 12, slight periportal F1-F2 = 17, extensive periportal F3 = 8, cirrhotic stage F4 = 6); activity was absent or weak (A0-A1) in 80% of cases. Advanced fibrosis F3-F4 (14 cases) was correlated with vacuolar changeG3 (27%) and G4 (50%). Liver carcinoma was present in 9 biopsies: 4 with extensive fibrosis (F3 or F4) and 5 without significant fibrosis (F0 = 2, F1 = 2, F2 = 1). Three cases had nodular regenerative hyperplasia (NRH). TEM observation showed mitochondrial lesions and absence of glycogen overload. IHC with αSMA revealed increased number of stellate cells in perisinusoidal and perivacuolar hepatocytes location.

Liver vacuolar change observed in 90% of ST with AP hyperactivity correlated with fibrosis stage (p = 0,051) and increased stellate cells activation suggested cellular dysfunction probably related to glucocorticoids metabolism. Cancer pathogeny remained to be explained.

Bibliography: Lecoindre P. *et al*. ECVIM Abstract, 2009 ; Center *et al*. JAVMA, 2006 ; METAVIR 1996.

## ABSTRACT HP-2

**CLINICAL FEATURES OF HEPATIC ENCEPHALOPATHY IN DOGS: 80 CASES (1991-2011).** JA Lidbury[1], R Ivanek[2], JS Suchodolski[1], JM Steiner[1]. [1]Gastrointestinal Laboratory and, [2]Veterinary Integrative Biosciences, Texas A&M University, College Station, TX

Hepatic encephalopathy (HE) is common in dogs, but few studies have specifically described this syndrome and the role of potential predisposing factors. The objectives of this study were to describe the clinical features of dogs with HE, to determine if there is any relationship between plasma ammonia concentrations and the severity of HE, and to determine what proportion of dogs with HE have any of the predisposing factors recognized in humans with HE, such as gastrointestinal hemorrhage, dehydration, or systemic inflammatory response syndrome (SIRS).

Medical records from all dogs with a recorded diagnosis of HE between 1/10/91 and 1/10/11 (20 years) were reviewed. The history, physical examination findings, results of diagnostic testing, and diagnoses from each case were recorded. The severity of HE was graded using a previously described system: grade I: apathy, decreased alertness, staring, and/or unawareness of surroundings; grade II: ataxia, circling, head pressing, blindness, and/or salivation; grade III: stupor, severe salivation, and/or completely inactive but arousable; and grade IV: coma and totally unresponsive. Plasma ammonia concentrations were compared amongst the groups of dogs with different grades of HE using a Kruskal-Wallis test. Significance was set at $p < 0.05$.

Eighty dogs met the inclusion criteria for the study. The most common historical findings were: obtundation in 26 dogs (33%), abnormal behavior in 23 (29%), head pressing in 22 (28%), ataxia in 21 (26%), and apparent seizures in 19 (24%). On neurological examination 46 dogs (58%) had abnormalities recorded. These included obtundation in 25 dogs (31%), ataxia in 16 (20%), weakness in 8 (10%), decreased conscious proprioception in 7 (9%), and seizures in 6 (8%). Twelve of 76 dogs (16%) for which it was possible to grade HE had grade I, 60 (79%) had grade II, 4 (5%) had grade III, and none had grade IV. Dogs with HE grades I, II, and III had median (range) plasma ammonia concentrations of 143 (50-356), 203 (15-617), and 361 (106-1350) μg/dL, respectively (p = 0.091). Forty-one of 65 dogs (63%) for which a cause of HE had been recorded had congenital portosystemic vascular anomalies, 16 (25%) had acquired portosystemic collaterals (APSC), 4 (6%) had hepatic parenchymal disease but no APSC could be detected, and 3 (5%) had hepatic parenchymal disease but were not evaluated for APSC. Forty-three of 80 dogs (54%) had no potential predisposing factors recorded, 9 (11%) had hypoglycemia, 7 (9%) had hypokalemia, 6 (8%) had SIRS, 5 (6%) had a clinically important coagulopathy or DIC, 5 (6%) had hyponatremia, 4 (5%) had concurrent intracranial disease, and 4 (5%) had azotemia.

Portosystemic shunting due to congenital portosystemic vascular anomalies or APSC was the cause of HE in at least 88% of dogs evaluated. There was a non-significant trend for the group of dogs with HE severe enough to cause stupor to have higher plasma ammonia concentrations than those with less severe HE. Potential predisposing factors, such as hypoglycemia, hypokalemia, or SIRS were present in 46% of patients and thus should be considered in dogs with HE.

## ABSTRACT HP-3

**LONG-TERM SURVIVAL OF DOGS (N = 597) WITH CONGENITAL OR ACQUIRED PORTOSYSTEMIC SHUNTING: 1980-2010.** SA Center, JF Randolph, KL Warner, KC Sapa, WE Hornbuckle, AE Yeager, NL Dykes, HJ Harvey, JA Flanders. College of Veterinary Medicine, Cornell University, Ithaca, NY

Surgical attenuation is preferred treatment for canine congenital portosystemic vascular anomalies (PSVA). However, success of long-term medical management of large canine PSVA cohorts and dogs with anicteric acquired portosystemic shunts (APSS) is undetermined.

A retrospective survival study of dogs with PSVA (extrahepatic [EPSVA, n = 378], intrahepatic [IPSVA, n = 105]) and anicteric APSS (n = 114) over 31 contiguous years at Cornell University is described. Follow-up (91.5% cases) was derived from medical records and contact with managing veterinarians and clients. Medical management consisted of dietary protein-restriction/modification, ± lactulose, ± metronidazole. Surgical PSVA attenuation was by suture ligation (n = 225/239) to tolerance. Age at diagnosis, survival of EPSVA and IPSVA treated surgically (n = 189 and 50, respectively) and medically (n = 166 and 46, respectively), and APSS survival were compared using Wilcoxon rank sum test, Kaplan-Meier survival curves, and two-sample survival tests (Gehran-Wilcoxon and Log Rank Tests), respectively with alpha $< 0.05$. Dogs dying without treatment were censored. Survivals (years) at 50th (range:75th-25th) percentiles are shown.

Median age (years) at diagnosis was significantly younger for PSVA (1[0.1-12]) vs APSS (4[0.12-12]), and IPSVA (0.5[0.1-5]) vs EPSVA (1.5[0.12-12]); $P < 0.00001$. EPSVA survival (all dogs: n = 334, 10[6.0-13]; Yorkshire Terriers: n = 91; 12[6-13]) was significantly longer than IPSVA: n = 104, 4.5(1.2-9); all $P < 0.00001$. There was no survival difference between surgically- or medically-treated EPSVA (all dogs; Yorkshire Terriers [n = 41 and n = 43, respectively]), and surgically- or medically-treated IPSVA. Of surgically treated dogs, 63 % EPSVA and 58% IPSVA also received medical treatment. APSS survival (9.0[4.6-11]) was significantly shorter than medically-treated EPSVA (all dogs: 10 [6-13]; Yorkshire Terriers:12.0 [6.0-13]), all $P < 0.04$.

## ABSTRACT HP-4

**SERUM BRANCHED-CHAIN AMINO ACID/TYROSINE RATIO (BTR) IN DOGS WITH LIVER DISEASES.** M Sakai, Y Ohori, M Nagamune, Y Sakamoto, T Watari, M Uechi. Nihon University, Department of Veterinary Medicine, Kanagawa, Japan

Branched-chain amino acids (BCAA; valine, leucine, isoleucine) are decreased and aromatic amino acids (AAA; tyrosine, phenylalanine) are increased in the blood of dogs with hepatic disorders and hepatic encephalopathy. The degree of hepatic parenchymal damage can be evaluated by calculating the Fischer ratio, the molar ratio of BCAA to AAA by HPLC. The molar ratio of BCAA to tyrosine ratio (BTR) can also be determined using enzymatically quantified values in humans with chronic liver disease.

We determined serum BTR for 92 dogs with chronic hepatitis (CH, n = 27), primary hyperplasia of the portal vein (PHPV, n = 15), congenital portosystemic shunts (CPSS, n = 41), and

hepatocellular carcinoma (HCC, n = 9) using the Diacolor-BTR kit. Acquired portosystemic collaterals (APSCs) were confirmed in 14 dogs with CH and in all dogs with PHPV. Plasma amino acids were analyzed by HPLC in 24 healthy dogs included as controls. The BTR closely correlated with the Fischer ratio in controls ($r = 0.89$, $p < 0.001$). The BTR was significantly lower in dogs with PHPV (median, 3.28) and CPSS (2.95) than in controls (10.62; $p < 0.001$). In addition, BTR was significantly decreased in CH dogs with APSCs (3.65) compared with controls ($p < 0.001$). In contrast, BTR was not significantly reduced in CH dogs without APSCs (6.17) and HCC dogs (8.71) as compared to controls.

The BTR is quite simple to determine by enzymatic methods in dogs and it is considered a useful indicator of liver parenchymal injury and for monitoring responses to nutritional therapy in dogs with CPSS and APSCs.

**ABSTRACT HP-5**
**PLASMA AND HEPATIC ENDOTHELIN-1 IN DOGS WITH CHRONIC LIVER DISEASES.** Y Sakamoto, M Sakai, T Watari. Nihon University, Department of Veterinary Medicine, Kanagawa, Japan

Elevated plasma endothelin-1 (ET-1) has been reported in human chronic liver diseases, particularly cirrhosis accompanied by esophageal varices. ET-1, which is a potently vasoactive, 21-amino acid peptide, has been evaluated for associations with respiratory and cardiac disorders, but not hepatic disorders, in dogs.

In this study, blood samples were collected from 12 dogs with chronic hepatitis/cirrhosis (CH). Liver biopsy samples were obtained laproscopically using 5-mm biopsy forceps from 12 dogs with CH and visually inspected for acquired portosystemic collaterals (APSCs). Plasma and hepatic ET-1 concentrations were determined using an ELISA kit, and hepatic ET-1 mRNA expression was evaluated using real-time PCR. Plasma (n = 14) and liver (n = 3) samples were obtained from healthy dogs as controls.

Plasma ET-1 concentration was higher in dogs with CH (median, 1.30 pg/mL; range, 0.68-2.65) than controls (median, 0.86 pg/mL; range, 0.41-1.41; $p < 0.05$), and hepatic ET-1 concentration was higher in dogs with CH (median, 11.78 pg/mL; range, 3.55-23.90) than controls (median, 2.72 pg/mL; range, 1.70-3.34; $p < 0.05$). In dogs with CH, confirmation of APSCs (n = 8) was associated with higher plasma ET-1 concentration. Hepatic ET-1 mRNA expression was significantly greater in dogs with CH than controls ($p < 0.05$).

The ET-1 system was shown to be an important factor in canine chronic liver disease. ET-1 may function as a potent vasoconstrictor agonist in intrahepatic blood flow regulation in CH and play a role in the pathogenesis of portal hypertension.

# SMALL ANIMAL - IMMUNOLOGY

**ABSTRACT IM-1**
**EFFICACY OF MYCOPHENOLATE MOFETIL FOR THE TREATMENT OF CANINE IMMUNE-MEDIATED HEMOLYTIC ANEMIA: 31 CASES (2007-2011).** A Wang, JR Smith. University of Georgia College of Veterinary Medicine, Athens, GA

This retrospective case series evaluated the clinical use and adverse effects of mycophenolate mofetil (MMF) for treating canine primary immune-mediated hemolytic anemia (IMHA).

Medical records (2007-2011) were searched for the diagnosis of IMHA (n = 93). Inclusion criteria included PCV < 35%, with either a positive slide agglutination test, spherocytosis, evidence of hemolysis or positive Coombs' test. Exclusion criteria included IMHA secondary to infectious causes, drug toxicity, or neoplasia (n = 29). Data collected included presenting complaints, signalment, vital parameters, body condition score, clinicopathologic data, diagnostic imaging results, medications administered, duration of hospitalization, survival time, and adverse effects.

Of 64 primary IMHA cases, 57 received steroids and a secondary immunosuppressive drug: 31 mycophenolate, 15 cyclosporine, 6 azathioprine, 1 human immunoglobulin. Seven dogs received three immunosuppressives; 2 received only steroids; 2 were immediately euthanized.

Data are presented as mean ± SD for dogs receiving mycophenolate mofetil. Initial PCV was 17 ± 5.2%. Initial MMF dose was 20.5 ± 5.8 mg/kg PO or IV divided twice daily. Diarrhea was the only adverse effect noted (n = 5), although one of these dogs presented with diarrhea. Number of transfusions received was 1.9 ± 1.6; hospitalization duration was 5.7 ± 4.5 days. Twenty-four dogs (77.4%) survived to discharge, 2 were lost to follow-up, and 18 were alive at study conclusion. Mean survival time was 13 days in non-survivors.

In conclusion, oral and IV administration of mycophenolate mofetil is a safe additional immunosuppressive for treating primary canine IMHA.

**ABSTRACT IM-2**
***EX VIVO* IMMUNOSUPPRESSION OF CANINE T LYMPHOCYTE-SPECIFIC PROLIFERATION USING DEXAMETHASONE, CYCLOSPORINE, AND THE ACTIVE METABOLITES OF AZATHIOPRINE AND LEFLUNOMIDE IN A FLOW CYTOMETRIC ASSAY.** LA Nafe, JR Dodam, CR Reinero. University of Missouri College of Veterinary Medicine, Columbia, MO

A high rate of mortality, expense and complications of immunosuppressive therapy in dogs underscores the need for optimization of drug dosing. While pharmacokinetics helps target therapeutic blood concentrations, pharmacodynamics measures the actual effects of the drug on the immune system. To date, no study has compared a panel of immunosuppressant drugs on T lymphocyte proliferation using a flow cytometric assay in the dog. The purpose of this study was to determine the 50% T cell inhibitory concentration (IC50) in dexamethasone, cyclosporine, and the active metabolites of azathioprine (6-Mercaptopurine) and leflunomide (A77 1726) treated concanavalin A-stimulated canine lymphocytes. Whole blood was collected from 7 privately owned, healthy dogs of various ages for density gradient separation of peripheral blood mononuclear cells (PBMCs). PBMCs were cultured for 72 hours with concanavalin A, a fluorochrome-marked cell proliferation dye and various concentrations of dexamethasone (-4, -5, -6 and -7 M), cyclosporine (10, 20, 30 and 40 ng/mL), 6-mercaptopurine (50, 100, 250 and 500 nM) and A77 1726 (5, 10, 25 and 50 uM). Following incubation, lymphocytes were labeled with propidium iodide and an anti-CD5 antibody. Flow cytometry determined the % live proliferating T lymphocytes with and without immunosuppressants. Mean ( SEM) IC50 were: dexamethasone, -4.6 0.5M; cyclosporine 16 2ng/mL; 6-mercaptopurine 64 31nM; and A77 1726, 24 3uM. Inhibition of T cell proliferation for four immunosuppressants was demonstrated in a concentration-dependant manner with marked variability between dogs. These results represent the first steps to tailor this assay for individual immunosuppressant protocols for dogs with immune-mediated disease.

# SMALL ANIMAL - INFECTIOUS DISEASE

**ABSTRACT ID-1**
***CYTAUXZOON FELIS* CYTOCHROME *B* GENOTYPE IS ASSOCIATED WITH SURVIVAL IN DOMESTIC CATS WITH CYTAUXZOONOSIS.** M Downey[1], HS Marr[1], J Tarigo[1], LA Cohn[2], MG Levy[1], AJ Birkenheuer[1]. [1]North Carolina State University College of Veterinary Medicine, Raleigh, NC, [2]University of Missouri College of Veterinary Medicine, Columbia, MO

*Cytauxzoon felis* is a virulent tick-transmitted protozoan parasite that infects felines. Without treatment, only 3% of infected cats survive. Treatment combining atovaquone and azithromycin (A&A) increased the survival rate to 60%[1]. In related parasites, resistance to atovaquone has been attributed to mutations in its

drug target, cytochrome *b* (*cytb*)[2]. We hypothesized that *C. felis cytb* genotypes were associated with response (i.e. survival) to A&A treatment. *Cytauxzoon felis cytb* genes were amplified by PCR from samples collected from 45 cats with cytauxzoonosis that received A&A treatment[1]. Amplicons were sequenced bi-directionally, chromatograms were inspected for heterozygosity and the sequences were edited accordingly[3,4]. The majority of samples (30/45) had evidence of a single *cytb* genotype, while the remaining samples (15/45) had two or more *cytb* genotypes present. The most common single genotype was arbitrarily assigned as the wild-type (WT). Two samples had missense mutations compared to the WT, but there was no association with survival or mortality. There was a significant difference ($p = 0.001$) between survival rates of cats infected with *C. felis* that solely contained the WT *cytb* (12/12 survived) compared to those infected with *C. felis* of any other *cytb* genotype (16/33 survived). However, the mere presence of the WT *cytb* (i.e. alone or concurrent with other genotypes) was not associated ($p = 0.35$) with survival (15/21 survived) when compared to cats without the WT *cytb* (13/24 survived). In conclusion, there is a high degree of genetic variability in the *C. felis cytb* gene, and *cytb* genotypes may be associated with survival.

**ABSTRACT ID-2**

**DIAMENAZINE DIACETURATE AT 3 MG/KG DOES NOT ELIMINATE PARASITEMIA IN CATS WITH CHRONIC *CYTAUXZOON FELIS* INFECTION.** KM Lewis[1], LA Cohn[1], H Marr[2], AJ Birkenheuer[2]. [1]University of Missouri – College of Veterinary Medicine, Columbia, MO, [2]North Carolina State University College of Veterinary Medicine, Raleigh, NC

*Cytauxzoon felis* is a hematoprotozoan parasite of cats that causes substantial morbidity and mortality, but cats that survive acute infection remain persistently parasitemic and may serve as a source of infection for vector ticks. We tested the ability of the antiprotozoal drug diminazene diaceturate to eliminate persistent parasitemia from seven apparently healthy cats with naturally acquired chronic *C. felis* infections.

Cats were administered either 3 mg/kg of drug or saline intramuscularly twice, one week apart. Cats that remained parasitemic after 10 weeks were crossed over to the other treatment and followed for an additional 10 weeks. Cats were observed for adverse reactions via periodic examination, CBC, plasma biochemical profile, and urinalysis. At 0, 3, 6, and 10 weeks after treatment pathogen number was assessed. Blood smears were prepared immediately, stained with Wright-Giemsa and examined thoroughly for piroplasms. Blood collected in EDTA was used for real time PCR for *C. felis*. Cycle threshold (Ct) was also used as a semi-quantitative measure of parasite burden in peripheral blood. Ct was compared between days using a two way repeated measures ANOVA.

Hypersalivation after injection was the major adverse reaction and was alleviated by atropine pre-treatment. Piroplasms were observed at low numbers on all blood smears at all time points and all blood samples were PCR positive for *C. felis*. Although 3 mg/kg diminazene was safely administered, it failed to eliminate or reduce parasitemia in chronically infected carrier cats.

**ABSTRACT ID-3**

**DETECTION OF LEPTOSPIRURIA IN SHELTER CATS IN COLORADO.** A Fenimore, K Carter, KF Lunn. Department of Clinical Sciences, Colorado State University, Fort Collins, CO

Leptospirosis is an important zoonotic bacterial disease affecting many mammalian species worldwide, including livestock, horses, dogs, and people. No studies have clearly documented naturally occurring clinical leptospirosis in cats, but experimental feline infections have been shown to result in leptospiruria. The purpose of this study was to use a quantitative real-time PCR (qPCR) assay to determine if pathogenic leptospires are shed in the urine of shelter cats.

Stray and feral shelter cats in Colorado having spent time outdoors were included in the study. Relinquished cats, indoor cats, and cats receiving antibiotics were excluded. At least 5 mL of voided urine was collected from each cat and DNA was isolated using the QIAamp Viral RNA Mini Kit®. For the qPCR, primers were used to amplify an 87 base pair product from a region of the *Leptospira spp*. 16S ribosomal DNA sequence. This assay is specific for pathogenic leptospires and has been validated in canine urine. To validate the qPCR for this study the assay was used in normal feline urine to which live pathogenic leptospiral serovars were added in serial 10-fold dilutions. The assay showed linearity over a range of dilutions and the threshold (Ct) was set at 36 cycles.

Urine samples were obtained from a total of 85 shelter cats; 10 cats were positive for leptospires in the urine based on the qPCR assay ($Ct < 36$).

Outdoor cats in Colorado can shed pathogenic leptospires in the urine and may potentially be reservoir or incidental hosts in the transmission of leptospirosis.

**ABSTRACT ID-4**

**IDENTIFICATION OF RHOPTRY ASSOCIATED PROTEIN-1S (RAP-1S) WITH BABSESIA GIBSONI ISOLATES OF INFECTED DOGS IN THE UNITED STATES.** MR Krecic[1], H Marr[2], AJ Birkenheuer[2]. [1]Orlando, FL, [2]College of Veterinary Medicine, North Carolina State University, Raleigh, NC

Babesia gibsoni organisms are obligate intraerythrocytic protozoa that infect dogs, often American pit bull terriers and American Staffordshire terriers in the United States (US). Babesia gibsoni infections are endemic in Asia, Africa, Europe, and portions of the Americas. Infection may cause fever and progressive anemia. Infected dogs that fight with susceptible dogs and infected ticks transmit B gibsoni sporozoites, leading to erythrocyte invasion. Rhoptry proteins are one of several believed to be necessary for parasite attachment and invasion of host erythrocytes. Antibodies against rhoptry associated protein-1s (RAP-1s) have been shown to protect cattle from infection by Babesia bigemina and Babesia bovis. Thus far, three RAP-1s; RAP-1 A, RAP-1 B, and RAP-1 C; from B gibsoni have been identified and DNA sequenced. North Carolina State University has identified two additional RAP-1s from B gibsoni that have not yet been reported and DNA sequenced. Goal of this project is to determine the presence of the three previously reported and the two new RAP-1s in isolates of B gibsoni from naturally infected dogs from the US.

We used primers developed at North Carolina State University for polymerase chain reaction (PCR) to confirm the presence of previously reported RAP-1 A, RAP-1 B, and RAP-1 C, and newly identified RAP-1 D and RAP-1 rhoptry related antigen (RRA) among B gibsoni isolates of ten naturally infected dogs diagnosed with babesiosis. We optimized PCR conditions such that the desired targets (i.e. RAP-1 A, RAP-1 B, etc) are amplified rather than dog genomic DNA. Optimal PCR conditions were 95°C for 20 seconds for DNA denaturing, 60°C for 30 seconds for primer annealing, and 72°C for 2 minutes for Taq polymerization. Forty-five PCR cycles were run.

RAP-1 A, RAP-1 B, RAP-1 C, RAP-1 D, and RAP-1 RRA were confirmed among B gibsoni isolates from these ten naturally infected dogs.

Next steps are to sequence the DNA for each RAP-1 identified with these B gibsoni isolates and determine their degree of conservation. If the DNA of at least one RAP-1 variant is highly conserved among isolates, they may be future targets for a diagnostic assay and a therapy.

**ABSTRACT ID-5**

**IRON STATUS AND C-REACTIVE PROTEIN IN CANINE LEISHMANIASIS.** P Silvestrini[1,2], A Zoia[1], M Planellas[2], X Roura[2], J Pastor[2], JJ Cerón[3], M Caldin[1]. [1]San Marco Veterinary Clinic, Padua, Italy;, [2]Autonomous University of Barcelona, Spain;, [3]University of Murcia, Spain

Previous studies demonstrated increased concentrations of acute phase proteins in dogs with canine leishmaniasis (CL).

The objective of the study was to investigate in dogs with CL the iron status and its relation with c- reactive protein (CRP), as sensitive marker of inflammation.

Iron, UIBC, TIBC, % of saturation, ferritin and CRP were measured in 86 dogs (group 0) presented to the San Marco Veterinary Clinic (November 2008-December 2011) and in which CL was diagnosed based on presence of clinical signs, high-titer anti-*Leishmania* antibodies and positive real-time PCR on bone marrow sample. All dogs were classified as stages C or D, according to the Canine Leishmaniasis Working Group guidelines. Control groups consisted of age, sex and breed-matched dogs from the same database. Group 1 (n = 86) were healthy patients and Group 2 (n = 86) were non-CL sick patients.

Dogs affected by CL showed significant lower ($p < 0.0001$) iron and TIBC and significant higher ($p < 0.0001$) CRP and ferritin concentrations respect to both control groups. UIBC and % of saturation of group 0 were significantly lower ($p = 0.02$ and $p = 0.0005$, respectively) only compared to group 1.

Within group 0 serum CRP was significantly correlated ($p < 0.0001$, $r = -0.46$) with serum iron. In this group 6/86 (6.9%) dogs died 28 days post-diagnosis. Their serum CRP was significantly higher than survival ($p = 0.03$)

To assess if plasma ferritin concentrations in dogs with CL was affected only by the degree of inflammation, the three groups were stratified based on different CRP concentrations. Plasma ferritin was found still significantly higher ($p < 0.001$) in group 0 compared to control groups.

Inflammation contributes to decrease plasma iron concentrations. Degree of inflammation is a risk factor for outcome. Increased ferritin is not only due to inflammation, but may reflect other specific mechanisms implicated in intracellular infections as demonstrated in human beings.

## ABSTRACT ID-6

## PREVALENCE OF *BLASTOCYSTIS* SPP AND *GIARDIA* SPP IN DOGS AND CATS RESIDENT IN A PACIFIC NORTHWEST SHELTER.

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*Blastocystis* spp and *Giardia* spp are protozoan parasites of the gastrointestinal tracts of many mammalian species. *Giardia* spp are well-recognized enteric pathogens in dogs, cats, and humans, while there is limited data regarding the prevalence of *Blastocystis* spp in companion animals. *Blastocystis* spp are, however, the most commonly recognized enteric protozoan parasite in human patients with gastrointestinal disease within the Pacific Northwest region of the USA. The aim of this study was to estimate the prevalence of these two organisms within a population of shelter-resident dogs and cats in the Pacific Northwest.

Freshly voided stool samples were collected from 40 dogs and 40 cats resident at the Oregon Humane Society facility in Portland, Oregon. *Giardia* carriage was assessed using a commercially available immunofluoresence assay kit (Merifluor® Cryptosporidium/Giardia), while *Blastocystis* spp were detected using a novel nested PCR methodology, following extraction of DNA from feces using routine methods. Amplified material from all samples testing positive for *Blastocystis* in the PCR assay was isolated and sequenced to confirm results. Contingency table analysis was used to compare the relative risk of carriage of *Giardia* spp and *Blastocystis* spp between dogs and cats.

*Giardia* spp were detected in stool samples from 6/40 dogs and 0/40 cats. Dogs were significantly more likely to test positive for *Giardia* than cats ($p < 0.05$, Fischer's Exact Test). *Blastocystis* spp were detected in stool samples from 9/40 dogs and 19/40 cats, with cats being significantly more likely to test positive for *Blastocystis* than dogs ($p < 0.05$, Fischer's Exact Test). Coinfection with both protozoa was noted in 2/40 dogs. Shelter resident animals overall were significantly more likely to test positive for *Blastocystis* spp than *Giardia* spp. ($p < 0.01$, Fischer's Exact Test).

We conclude that both *Giardia* spp and *Blastocystis* spp are common in shelter-resident animals in the Pacific Northwest region. Cats apparently have a higher carriage rate of *Blastocystis* than dogs, however, in this population of cats at least, *Giardia* spp were uncommon.

## ABSTRACT ID-7

## TREATMENT OF CHRONIC RHINITIS IN SHELTER CATS WITH PARENTERAL ALPHA-INTERFERON OR AN INTRANASAL FELINE HERPESVIRUS 1 AND FELINE CALICIVIRUS VACCINE.

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Feline upper respiratory tract disease is a leading cause for euthanasia in shelter cats and can be frustrating to treat. The purpose of this study was to evaluate novel treatments for cats from shelters with suspected chronic viral rhinitis that have failed conventional therapy.

Cats from shelters that failed traditional therapy (lysine; antibiotics) for 2-3 weeks were transported to CSU and randomly assigned to one of two treatment groups. Group A was administered human interferon alpha (10,000 Units/kg SQ) once daily for 14 days. Group B was administered an intranasal FHV-1/FCV vaccine (HESKA Corporation, Loveland, CO) on Day 1 as immunotherapy followed by 1 mL of saline SQ once daily for 14 days. A clinical score for each cat was determined daily by a trained, blinded individual. Cats who improved (clinical score $< 3$) by day 14 were eligible for adoption. Cats with a score of $\geq 3$ on Day 14 were entered into the crossover group. Cats who failed both therapies and/or had severe ocular signs were administered famciclovir as a rescue drug for FHV-1. Total DNA and RNA was extracted from nasal or pharyngeal swabs collected prior to the first treatment and evaluated for the presence of FHV-1 DNA and FCV RNA using previously reported molecular assays.

A total of 47 cats were transferred from the shelters to the research facility to enter the treatment study. Of these cats, 16 had clinical signs resolve spontaneously during the final equilibration period before treatment, three cats required famciclovir treatment immediately due to ocular ulcers, one cat had to be euthanized prior to treatment because of severe stomatitis, and one cat was euthanized early during the first treatment period because of severe stomatitis. One cat had a clinical score $> 3$ after both treatments and famciclovir rescue therapy and was found on workup to have a severe proliferative rhinitis. All other cats ($n = 25$) had a clinical score of $< 3$ during the first treatment period or soon after crossover. Response rates to primary treatment with interferon alpha or intranasal vaccine administration were 66.7% (8 of 12 cats) and 100% (13 of 13 cats), respectively ($p = 0.039$). All four cats with clinical scores $> 3$ after alpha interferon therapy had clinical scores $< 3$ two to seven days after the crossover to intranasal vaccine administration. Mean time to response for those cats developing clinical scores $< 3$ during the first treatment was not significantly different between groups. All six cats positive for nucleic acids of FHV-1, FCV, or both responded to the primary treatment (2 cats-interferon alpha; 4 cats-intranasal vaccine).

Administration of human interferon alpha or a topical FHV-1/FCV vaccine as immunotherapy may be beneficial in alleviating chronic clinical signs of suspected feline viral URTD in some cats.

## ABSTRACT ID-8

## INTRANASAL ADMINISTRATION OF A MODIFIED LIVE FELINE HERPESVIRUS 1 AND FELINE CALICIVIRUS VACCINE INDUCES CROSS PROTECTION AGAINST *BORDETELLA BRONCHISEPTICA*.

A Bradley[1], J Kinyon[2], T Frana[2], D Bolte[3], DRHyatt[3], MRLappin[1]. [1]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, [2]Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA, [3]Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, CO

Vaccination against all feline upper respiratory pathogens is not possible. Results of previous studies suggest that intranasal vaccination may stimulate nonspecific immunity against agents not contained within the vaccine, but no study has directly examined this in cats. The authors hypothesized that cats administered a modified live feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV) intranasal vaccine would have fewer clinical signs after challenge inoculation with *Bordetella bronchiseptica* than unvaccinated controls.

Twenty specific pathogen free 12 week-old kittens were randomized into 2 groups of 10 cats each. The vaccinated group was administered a single intranasal dose of a commercially available vaccine containing modified live strains of FHV-1 and FCV, and the control group remained unvaccinated. All 20 cats were administered *B. bronchiseptica* by nasal inoculation seven days later and were observed daily for clinical signs of illness for 20 days.

In the first 10 days after *B. bronchiseptica* challenge, vaccinated cats were less likely to be clinically ill (indicated by lower cumulative clinical scores) than control cats ($p = 0.01$). The most commonly observed clinical sign was sneezing. Overall, 9 of 10 control cats and 2 of 10 vaccinated cats were noted to sneeze at least once during days 1-10 after inoculation with *B. bronchiseptica* ($p = 0.006$). These differences were no longer apparent during days 11-20. Finally, the percentage of observation points with sneezing recorded was significantly greater in control cats than in vaccinated cats over days 1-10 ($p < 0.0001$) and days 1-20 ($p = 0.02$).

Intranasal vaccination against FHV-1 and FCV decreased signs of illness due to an infectious agent not contained in the vaccine. This nonspecific immunity could be beneficial for protection against organisms for which vaccines are not available and as early protection while specific adaptive immunity is developing.

## ABSTRACT ID-9

## FELINE HERPESVIRUS 1 VIRAL LOAD AS A TOOL TO DIFFERENTIATE LYTIC FROM LATENT INFECTION.

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Feline Herpesvirus 1 (FHV-1) detection by real-time polymerase chain reaction (PCR) is considered the most sensitive diagnostic tool in feline upper respiratory infections (URI). It has long been criticized that PCR overestimates FHV-1 prevalence because of detecting latent virus not involved in causing respiratory disease manifestation. In this report we review three studies that establish protocols to use FHV-1 DNA viral load to differentiate acute, lytic from latent infections.

In the first study, FHV-1 experimentally infected cats were monitored with clinical examination, quantitative virus isolation and quantitative real-time PCR. Viral load by PCR correlated with quantitative virus isolation during the clinical phase but remained detectable at a lower level for a period of 8 weeks while cats were free of clinical signs. A PCR cutoff value was established to differentiate virus loads associated with clinical signs and positive virus isolation indicating lytic virus from later stages in infection with negative virus isolation, absence of clinical signs characterizing the chronic or latent stage of FHV-1 infection. In a second study, immune induction and FHV-1 replication was studied in shelter cats naturally infected with FHV-1. Cats with confirmed FHV-1 infection by virus isolation had a specific type of immune induction, detectable FHV-1 replication and high DNA viral loads measured by real-time PCR. In a third study, a link was established between lytic FHV-1 infection in naturally infected shelter cats, herpes specific inclusions, histological scores in biopsies, and FHV-1 DNA load measured by real-time PCR. This study was able to establish causality between the presence of FHV-1 and disease manifestation and confirmed that FHV-1 DNA viral load correlated with lytic FHV-1 infection.

In conclusion, these studies strongly indicate a link between FHV-1 DNA viral load and clinical parameters, viral replication, isolation in cell culture, immune reaction, pathognomonic inclusions, and histological grades for tissue damage. Real-time PCR therefore could be a key tool to differentiate between lytic and latent FHV-1 infection in cats and could be used to determine if FHV-1 is the cause of URI in individual cats.

## ABSTRACT ID-10

## SERIAL FIV SEROLOGICAL RESULTS IN COHABITING FIV-POSITIVE AND FIV-NEGATIVE CATS.

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Horizontal transmission of feline immunodeficiency virus (FIV) most commonly occurs via the inoculation of infected saliva into bite wounds during territorial fights. Conflicting accounts have been published in the veterinary literature regarding transmission between cohabiting cats in stable closed households and the mechanics of possible casual transmission, if it occurs, are poorly understood. The aim of this study was to document the FIV serological status of cats living long-term in a stable multi-cat household containing FIV-positive and FIV-negative cats.

Serological testing (IDEXX SNAP FIV/FeLV Combo) was performed on 138 cats living in a stable multi-cat household. All cats had unrestricted access to each other. Eight of the 138 cats were FIV-positive (6 MN, 2 FS; median age 28 months; range 5-129 months) and 130/138 cats were FIV-negative (71 MN, 59 FS; median age 4 months; range 2-155 months). Some inter-cat fighting took place, mainly involving 2 cats known to be FIV-positive, but only scratches were observed rather than bite wounds. All cats were kept indoors only, except for 1 FIV-positive cat, which was housed indoor/outdoor. No cats were FeLV-positive on the first test.

Testing was repeated in 50/138 and the FIV/FeLV serological status of each cat was unchanged, (FIV-positive $n = 5$; FIV-negative $n = 45$; median 28 months after the first test; range 1-106 months), except for one of the FIV-negative cats, which was FeLV-positive on the second test. That cat was transferred to another household 6 days after the test and subsequently died 2 weeks later. A third test was performed on 5/138 cats (median 3 months after the second test; range 1-45 months) and FIV and FeLV serological status remained unchanged (FIV-positive $n = 1$; FIV-negative $n = 4$). Total cumulative exposure to FIV-positive cats was calculated for each FIV-negative cat that had at least 2 test results and was exposed to FIV-positive cats (months of exposure to each FIV-positive cat was calculated and the results for the FIV-negative group were added; FIV-positive $n = 5$; FIV-negative $n = 44$; median cumulative exposure duration of FIV-negative cats to FIV-positive cats = 140 months; range 14-167 months)

It is concluded that despite occasional minor fighting between FIV-positive and FIV-negative cats kept in a stable multi-cat household, FIV transmission did not occur over years of cumulative exposure to FIV-positive cats.

## ABSTRACT ID-11

## U. S. PREVALENCE OF CANINE INFECTIOUS RESPIRATORY DISEASE PATHOGENS: A 3-YEAR STUDY.

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The diagnosis of canine infectious respiratory disease (CIRD) is complicated by the emergence of at least four canine respiratory pathogens since 2000: canine influenza virus (CIV), canine respiratory coronavirus (CRCoV), *Mycoplasma cynos*, and *Streptococcus equi* subspecies *zooepidemicus*.

Pfizer established an Immunization Support Guarantee (ISG) program to diagnostically evaluate samples from dogs vaccinated with a Pfizer Animal Health vaccine that subsequently developed clinical signs of CIRD. Resulting data were combined with CIRD diagnostic data from Idexx Laboratories to determine the prevalence of nine common CIRD pathogens, including the four newly emerging agents. The combined databases included diagnostic results from 2008-2011 for >13,000 dogs from all 50 states.

The three most frequently identified pathogens in the Idexx database were *M. cynos* (36.5%, 682/1,867), *Bordetella bronchiseptica* (Bb) (24.2%, 3,247/13,429), and canine distemper virus (19.2%, 2,578/13,429). In the ISG database, the three most frequent diagnoses were *M. cynos* (59.6%/65/109), CRCoV (15.3%, 50/327), and Bb (14.4%, 47/327). Divergence in prevalences between the two databases can be partly explained by a high percentage of unvaccinated, at-risk shelter dogs in the Idexx database.

Results indicated that newly emerging CIRD pathogens may be the cause of CIRD in dogs that were vaccinated for conventional agents, and that *M. cynos* should be a prime suspect in clinical CIRD, especially in dogs that do not respond to antimicrobial therapy that targets other pathogens.

**ABSTRACT ID-12**

**ARTHROPOD-BORNE DISEASE IN SICK SOUTHERN CALIFORNIA DOGS.** L Kidd[1], M Barr[1], M Lappin[2], E Breitschwerdt[3], C Osmond[4], J Hart[5], S Hill[5], K Richter[5]. [1]Western University of Health Sciences, Pomona, CA, [2]Center for Companion Animal Studies, Fort Collins, CO, [3]Vector Borne Disease Diagnostic Laboratory, Raleigh, NC, [4]California Veterinary Specialists, Carlsbad, CA, [5]Veterinary Specialty Hospital, San Diego, CA

The distribution of arthropod borne disease (ABD) is expanding. Dogs are sentinels for ABD in people. Rocky Mountain spotted fever is rare in southern California due to a lack of *Dermacentor* ticks, the primary vector for *Rickettsia rickettsii* in North America. *Rhipicephalus sanguineus* caused recent outbreaks in Arizona and Mexicali, Mexico. This ubiquitous tick is also a known or suspected vector for *Ehrlichia*, *Babesia*, and *Bartonella* species. *Ixodes pacificus*, which transmits *Anaplasma phagocytophilum* and *Borrelia burgdorferi*, is also present in southern California. Studies investigating the prevalence of ABD in dogs in the southernmost regions of California are very limited. The objective of this study was to determine the prevalence of exposure to, or active infection with, ABD agents in ill dogs presenting to specialty hospitals in San Diego County.

Dogs presenting to three specialty hospitals in San Diego County with unexplained fever, anemia, thrombocytopenia, polyarthritis, ocular inflammation, epistaxis, myalgia, proteinuria, hyperesthesia, CNS signs, petechiae, vasculitis, cough, or GI signs with one other clinical sign were eligible for inclusion. Dogs receiving tetracycline antibiotics were excluded. DNA extracted from whole blood was tested using PCR for spotted fever group (SFG) *Rickettsia*, *Bartonella, Babesia, Ehrlichia, Anaplasma* and haemotropic *Mycoplasma*. Serum was tested for antibodies to *A. phagocytophilum, E. canis* and *B. burgdorferi* using ELISA (SNAP®4Dx®), and for antibodies to *R. rickettsii*, *E. canis, B. henselae, B. vinsonii ssp. berkhoffi*, and *B. canis* using immunofloresccent assays (IFA). Convalescent serum was tested for *R. rickettsii* antibodies (IFA).

Evidence of infection or exposure to one or more agents was present in 8 of 49 dogs (16.3%). *Ehrlichia canis* antibodies (IFA) and DNA were detected in 2 dogs; one of these dogs was also seropositive by SNAP® 4Dx®. One dog was PCR positive but seronegative for *B. canis*. One dog was PCR positive for *B. conradae, M. haematoparvum and M. haemocanis*, and seropositive for *B. vinsonii ssp. berkhoffi*. Antibodies to *A. phagocytophilum*, and *B. burgdorferi* were detected in one dog each. No dog tested PCR or IFA positive for SFG *Rickettsia* acutely. Convalescent titers of 1:64 to *R. rickettsii* were detected in 2 of the 31 dogs tested. One had cephalosporin responsive discospondylitis. The other had fever and arthralgia for 24 hours that resolved without treatment.

This study documents that exposure to or infection with ABD is relatively common in sick dogs presenting to specialty practices in this region of the United States. The weakly positive convalescent titers to *R. rickettsii* may have been due to cross-reacting antibodies rather than infection with SFG *Rickettsia*. However, clinicians should remain vigilant given the presence of competent vectors and proximity to outbreaks. This study also illustrates that limiting testing to either PCR or serology may overlook infection or exposure to ABD in some patients.

**ABSTRACT ID-13**

**NT-PCNP AS A DIAGNOSTIC BIOMARKER FOR SEPSIS IN DOGS PRESENTING FOR EMERGENCY CARE.** N Etedali, CR Sharp, M Burns, J Morlock. Tufts Cummings School of Veterinary Medicine, North Grafton, MA

In a single center study, N-terminal pro-CNP (NT-pCNP) has been shown to have high sensitivity and specificity (using a cut-off value of 10.1pmol/L) for the diagnosis of nonperitoneal sepsis and its differentiation from noninfectious causes of the systemic inflammatory response syndrome (nSIRS) in dogs admitted to an ICU. The purpose of this prospective serial admission study was to evaluate the diagnostic utility of NT-pCNP in a larger, more diverse, group of dogs presenting for emergency care. A secondary objective of the study was to further assess NT-pCNP in dogs with septic peritonitis. Blood samples were collected from dogs at the time of emergency room presentation for measurement of NT-pCNP. NT-pCNP was measured on serum with a previously validated ELISA. One hundred and eighty eight dogs were enrolled. Based on the deLaforcade SIRS criteria, nine dogs (5.05%) had confirmed sepsis, 12 (6.7%) had suspected sepsis, 56 (31.5%) had nSIRS, 9 had infection (5.05%) and 92 (51.7%) had no evidence of SIRS or infection. A high pCNP (above the cut-off) had a high specificity (0.92) but low sensitivity (0.42) for the diagnosis of sepsis in this population of dogs. Contrary to previous findings, NT-pCNP was a useful diagnostic biomarker for septic peritonitis in this study. Further work is needed to improve the diagnostic utility of NT-pCNP, notably to improve specificity, across a broad population of dogs. This may involve reassessment of the cut-off value and/or combination with other biomarkers.

**ABSTRACT ID-14**

**SERIAL NT-PCNP IN HOSPITALIZED DOGS WITH SEPSIS.** M Burns, CR Sharp, N Etedali, J Morlock. Tufts Cummings School of Veterinary medicine, North Grafton, MA

N-terminal pro-CNP (NT-pCNP) has been shown to have high sensitivity and specificity (using a cut-off value of 10.1pmol/L) for the diagnosis of sepsis and its differentiation from noninfectious causes of the systemic inflammatory response syndrome, when excluding dogs with septic peritonitis. The purpose of this prospective pilot study was to evaluate the utility of serial measurement of NT-pCNP in assessing response to treatment and assigning prognosis to hospitalized dogs with sepsis. A secondary objective of the study was to further assess NT-pCNP in dogs with septic peritonitis. Serial blood samples were collected from dogs with sepsis at hospital admission, and then at 24-hour intervals until hospital discharge for measurement of NT-pCNP. Fourteen dogs with sepsis were enrolled. The origin of sepsis was intra-abdominal (n = 5), pneumonia (3), urosepsis (3), cutaneous (2), and chemotherapy induced (1). Thirteen dogs had community acquired (CA) sepsis, while two dogs had hospital acquired (HA) sepsis. Thirteen dogs survived to discharge, and two were euthanized. All but one dog had an NT-pCNP concentration above the cut-off value. The two dogs with HA-sepsis had low NT-pCNP concentrations initially, that subsequently increased with the onset of sepsis. There was no predictable change in NT-pCNP over time in dogs with CA-sepsis. Despite a clinical response to treatment, NT-pCNP concentrations did not reliably decrease. NT-pCNP may not be a useful standalone biomarker to assess response to treatment in dogs with sepsis. Contrary to previous findings, NT-pCNP was a useful diagnostic biomarker for septic peritonitis in this study.

**ABSTRACT ID-15**

**PHYLOGENETIC DIVERSITY OF BACTERIA ISOLATED FROM SICK DOGS USING THE BAPGM ENRICHMENT CULTURE PLATFORM.** AC Davenport, PE Mascarelli, RG Maggi, EB Breitschwerdt. North Carolina State University, Raleigh, NC

It is estimated that fewer than 5% of bacterial organisms have been isolated using contemporary bacteriologic methods; however, isolation remains the gold standard for the diagnosis of bacterial infections. During the last decade, our laboratory developed and optimized a liquid growth medium, *Bartonella* alpha-Proteobacteria growth medium (BAPGM), in an attempt to more successfully isolate *Bartonella* spp. As BAPGM is not a selective medium, non-*Bartonella* spp. isolated from canine blood, pathological effusions, and tissue samples were phylogenetically characterized by 16S rDNA sequencing. The purpose of this study is to describe the phylogenetic bacterial diversity and disease manifestations in dogs infected with these isolates.

Non-*Bartonella* BAPGM subculture isolates were obtained from 70 of 513 dogs (14%) tested using the BAPGM platform. All isolates (n = 98) were members of 4 Phyla: 55 Proteobacteria, 26 Firmicutes, 14 Actinobacteria, and 3 Bacteroidetes. Members of the class alpha-Proteobacteria were more frequently isolated (n = 36) than any other bacterial class. Fourteen dogs were co-infected with a *Bartonella* spp. Retrospective review of the 26 dogs with NCSU-

VTH medical records identified *Sphingomonas* spp. (n = 7) and *Staphylococcus* (n = 5) as the most common isolates. These twenty-six dogs had variable clinical presentations, including 7 dogs with suspected immune-mediated disease, 4 with pathological effusions, and 4 with endocarditis. Direct comparison of conventional and BAPGM blood culture was not performed in this population; however, review of blood cultures performed for NCSU-VTH patients revealed the distribution of organisms isolated differed substantially: 58% Firmicutes, 24% Proteobacteria, 12% Actinobacteria, and 6% fungi using conventional culture techniques versus 63% Proteobacteria, 22% Firmicutes, 11% Actinobacteria, and 4% Bacteroidetes using BAPGM.

BAPGM preferentially facilitates growth of alpha-Proteobacteria. Future studies are needed to elucidate the diagnostic utility of BAPGM and other "non-conventional" growth media for the isolation of fastidious organisms from patients with immune-mediated, effusive and other "sterile" diseases and to determine if these organisms play a causal role in disease development.

## ABSTRACT ID-16

## PREVALENCE OF *BARTONELLA* SPP. AND HEMOPLASMAS IN THE BLOOD OF DOGS AND THEIR FLEAS IN FLORIDA.

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Fleas are known to be the vector for a number of *Bartonella* spp. and are believed to be associated with the transmission of some hemoplasmas. While a number of studies have evaluated dogs for these potential pathogens, little is known about prevalence rates in fleas. The objective of this study was to define the prevalence of these organisms in healthy shelter dogs and their fleas in Florida.

Blood (EDTA), serum, and fleas of unknown genus and species were collected from 37 healthy dogs on admittance to a shelter in Florida as part of routine screening for *Dirofilaria immitis* and tick borne diseases. The samples were stored at -20°C until shipped to Colorado State University. Upon arrival, total DNA was extracted from the blood and the fleas and assayed in previously reported genus-specific, conventional PCR assays that amplify the DNA of *Bartonella* spp. and hemoplasmas. An average of 4.8 fleas were collected from each dog; for the 4 dogs with > 5 fleas, the fleas were assayed in groups of 3 to 6. Positive amplicons were sequenced to confirm the species.

*Bartonella* spp. or hemoplasma DNA was amplified from 6 of 37 dogs (16.2%) and from at least one flea group from 5 of the dogs (13.5%) giving a total of 8 positive (dog, fleas, or both) sample pairs (Table 1). *Bartonella vinsonii* subsp. *berkhoffii* DNA was amplified most frequently.

While *B. vinsonii* subsp. *berkhoffii* DNA has been amplified from fleas (predominately *Pulex simulans*) collected on gray foxes, to our knowledge, this is the first study to amplify DNA of the organism from fleas collected from domestic dogs. In Florida, *C. felis* is the most prevalent flea feeding on dogs. The genus and species of the fleas are being determined for all new cases in this ongoing, prospective study. Results of this study document that flea-associated, potentially zoonotic, infectious agents are common in dogs and fleas in Florida and support the recommendation that stringent flea control be maintained on dogs.

**Table 1.**

| | Sample Pair # | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Blood | BV | NEG | BV | BV | BV, MH | BV | NEG | MHP |
| Fleas | NEG | BC | NEG | BV | BV | NEG | BV | BV |

NEG = negative; MH = *Mycoplasma haemocanis*; MHP = '*Candidatus* M. haematoparvum'; BV = *B. vinsonii*; BC = *B. clarridgeiae*.

## ABSTRACT ID-17

## CLINICOPATHOLOGIC FEATURES AND ATYPICAL PRESENTATIONS OF NATURALLY OCCURRING CANINE LEPTOSPIROSIS: 51 CASES (2000-2010).

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The purposes of this study were to retrospectively review the clinicopathologic presentations of dogs infected with leptospirosis, identify antibodies against *Leptospira* serovars based on acute and convalescent microscopic agglutination test (MAT), identify and describe the percentage of atypical abnormalities, and evaluate the importance of convalescent MAT titers.

Medical records of 138 dogs with leptospirosis presented to the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania between January 1, 2000 and January 1, 2010 were retrospectively reviewed. The MAT titers were considered positive when ≥1:1600 in vaccinated dogs, ≥1:800 in non-vaccinated dogs, or when demonstrating a ≥ 4-fold increase in convalescent titer.

Fifty-one dogs met the criteria for inclusion. Clinical presentation, temporal distribution and signalment were similar to previous studies. Convalescent MAT titers were necessary for diagnosis in 45% of cases. *L. icterohemorrhagiae* and *L. grippotyphosa* were the most commonly identified infectious serovars on MAT. Atypical abnormalities included hepatic dysfunction alone (22%), coagulopathy (29%), radiographic evidence of pulmonary disease (43%), proteinuria (82%), glucosuria (18%), and hypoalbuminemia (27%). Significant associations were found between *L. grippotyphosa* and renal involvement ($p = 0.02$), and *L. icterohemorrhagiae* and hepatic involvement ($p = 0.01$).

Atypical presentations were common. Awareness of atypical presentations may decrease misdiagnosis of leptospirosis for other disease entities (e.g. Lyme nephritis, protein-losing disease, tick-borne disease, pulmonary disease). Convalescent titers are necessary to identify infection when acute titers are negative. Further research is needed to determine the true association between infecting serovars and clinical presentation.

## ABSTRACT ID-18

## PARASITE BURDEN DOES NOT FLUCTUATE WITH TIME OF DAY IN CATS WITH CHRONIC *CYTAUXZOON FELIS* INFECTION.

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*Cytauxzoon felis* is a hematoprotozoan parasite of cats that causes substantial morbidity and mortality, but cats that survive acute infection may remain persistently parasitemic and may serve as a pathogen source for vector ticks. For many hematologic infections, parasite numbers in circulation vary markedly over periods of time as brief as hours, and often parasite numbers vary with time of day. If this were true for *C. felis*, it could impact a veterinarian's ability to identify chronically infected carrier cats.

Parasite burden was estimated in the peripheral blood of five apparently healthy cats with naturally acquired chronic *C. felis* infections. Peripheral blood was obtained twice in a 24 hour period, 12 hours apart, from each cat via jugular venipuncture on 4 separate occasions. In total, 20 samples were obtained at approximately 9 am and 20 samples obtained at approximately 9 pm into EDTA containing Vacutainer tubes. Blood smears were prepared immediately, and the reminder of the blood was used to perform CBC in routine fashion and for real time PCR for *C. felis*. Each slide was stained with Wright-Giemsa and examined thoroughly for piroplasms, and the number of piroplasms per 1500 RBC was noted. Cycle threshold (Ct) was also used as a semi-quantitative measure of parasite burden in peripheral blood. Ct was compared between times of day using a two way repeated measures ANOVA (two factor repetition).

Piroplasms were observed at low numbers on all blood smears at all time points, and all blood samples were PCR positive for *C. felis*. While at least a single RBC containing piroplasm was seen on every slide, the number of piroplasm infected RBCs per 1,500 RBC ranged from 0 to 4, with no difference between samples obtained in the morning versus the evening. The mean Ct for all cats on samples obtained in the morning was 24.1 ± 1.2

and the mean in the evening was 24.7 ± 2.0. No significant difference was detected in the Ct between samples obtained in morning versus the evening ($p = 0.107$). However, parasite burden was observed to vary from day to day in a given cat ($p = 0.041$). This suggests that timing of blood collection to detect the carrier state is unimportant despite some variability in parasite burden from day to day. PCR is a more sensitive method for detection of the carrier state, but if blood smear analysis is used, the entire smear must be carefully evaluated as piroplasms often occur in extremely low numbers.

**ABSTRACT ID-19**

**MITOCHONDRIAL GENOME SEQUENCES RESULT IN IMPROVED UNDERSTANDING OF THE PHYLOGENETIC RELATIONSHIPS OF PIROPLASMIDA THAT INFECT COMPANION ANIMALS.** M Downey[1], HS Marr[1], J Tarigo[1], LA Cohn[2], DM Bird[3], EH Scholl[3], MG Levy[1], AJ Birkenheuer[1]. [1]North Carolina State University College of Veterinary Medicine, Raleigh, NC, [2]University of Missouri College of Veterinary Medicine, Columbia, MO, [3]North Carolina State University College of Agriculture and Life Sciences, Raleigh, NC

Based on phylogenetic analyses using 18S ribosomal ribonucleic acid (rRNA) gene sequences, parasites in the order *Piroplasmida* have been sub-categorized into at least five distinct groups[1]. Piroplasma from these groups infect common companion animal species—dogs and cats. In this study, we characterized the mitochondrial genome sequence and structure from these piroplasma. We hypothesized that use of mitochondrial gene sequences would improve upon previous phylogenetic analyses that used 18S rRNA genes alone. Blood was collected from dogs or cats infected with species representing the proposed sub-groups: *Babesia microti*-like sp. (AKA, *Theileria annae*), *B. conradae*, *B. canis canis, B. canis rossi, B. canis vogeli*, an un-named *Babesia* sp. ("Coco"), and *Cytauxzoon felis*. DNA was isolated and the entire mitochondrial genome (5.6-5.9 kb) was PCR-amplified in overlapping fragments. Amplicons were sequenced bi-directionally, and contigs were assembled using a commercially available software package[2]. Mitochondrial genomes were annotated for *cox1*, *cox3*, *cytb* and ribosomal subunit genes. Phylogenetic trees were constructed using both mitochondrial genes and 18S rRNA gene (Neighbor-joining, 1000 bootstrap replicates[3]). Utilization of mitochondrial genes improved bootstrap support and resolved uncertainties concerning the relationship between *Cytauxzoon* species and *Theileria* species compared to trees based on 18S gene sequence alone. Additionally, one sub-group characterized by its tropism for ungulid hosts was shown to include a species (*Babesia* sp. "Coco") that parasitizes dogs. In conclusion, we demonstrate the utility of the mitochondrial genome structure and sequence for phylogenetic analyses, and propose that future studies include mitochondrial genes to further define evolutionary relationships between *Piroplasmida* species.

**ABSTRACT ID-20**

**PREVALENCE OF *BARTONELLA SPP.* ASSOCIATED CANINE INFECTIOUS ENDOCARDITIS IN BANGKOK, THAILAND.** S Surachetpong[1], S Assarasakorn[1], AV Scorza[2], MM Brewer[2], KW Simpson[3], MRLappin[2]. [1]Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand, [2]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, [3]Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY

Infectious endocarditis (IF) is a heart valve or endocardial disease caused by bacterial infection. *Bartonella* spp. is considered one of the major causes of IF. Stray dogs in Thailand were found seropositive to *Bartonella spp*. The prevalence of *Bartonella spp*. associated canine IF in Thailand has not been investigated.

The prevalence of IF was studied by the necropsy reports of dogs, from January 1999 to December 2009. The cardiac paraffin embedded tissue blocks were used to study the presence of *Bartonella* spp. by fluorescence *in situ* hybridization (FISH) and conventional polymerase chain reaction (PCR).

A review of 3545 dogs demonstrated 23 cases of IF, yielding a prevalence rate of 0.65%. Paraffin blocks from 11 dogs were retrieved. Two of 11 dogs were positive to eubacteria by FISH. None of these dogs was positive for *Bartonella spp*. by FISH and conventional PCR.

The prevalence of canine IF is low. *Bartonella spp*. was not detected in the dogs tested in this study. Other types of bacteria should be considered as the cause of IF in Thailand.

**ABSTRACT ID-21**

**PREVALENCE OF SELECTED INFECTIOUS DISEASES IN SAMOAN DOGS.** RJ Carslake[1], KE Hill[1], K Sjölander[2], D Prattley[1], E Acke[1]. [1]Institute of Veterinary and Biomedical Sciences, Massey University, Palmerston North, New Zealand, [2]Faculty of Veterinary and Animal Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

Samoa has a tropical island climate ideally suited to many infectious diseases, and vectors for some infectious diseases are known to be present. Dogs are very common pets in Samoa with 88% of households owning an average of two dogs, which live in direct contact with their owners and the wider village community. These dogs also come into close proximity to a substantial tourist population visiting this holiday destination. Many canine infectious diseases are zoonotic and there is limited preventative medicine available for dogs in Samoa. There are very few studies into the presence of zoonotic pathogens in Samoa or other South Pacific islands, and the role of dogs as a reservoir for zoonotic diseases is unknown.

The prevalence of selected infectious diseases was evaluated in 242 dogs undergoing surgical sterilisation in Samoa in July 2010 and August 2011. Dogs were selected from both main Samoan islands, in rural and urban areas. Data were obtained from dogs' owners by interview, including age, environment and any previous preventative medication. Serum and fecal samples were collected, and the skin examined for external parasites. Seroprevalence of five vector-borne diseases were assessed in 237 dogs using point of care qualitative ELISA assays (SNAP Leishmania and SNAP 4Dx, IDEXX, Westbrook, ME) to detect antibodies against *Leishmania infantum, Anaplasma phagocytophilum, Ehrlichia canis* and *Borrelia burgdorferi,* and *Dirofilaria immitis* antigens. Fecal analysis was performed by flotation testing on fresh fecal samples from 204 dogs to screen for intestinal parasites. Forty-eight fecal samples were tested for *Giardia* and *Cryptosporidium* spp.

The median age of dogs enrolled was one year, with a range of four months to eight years and 73.3% (176/240) were male. The vast majority of dogs, 95% (230/242), were owned, the remaining were stray animals. The seroprevalence of *D. immitis* was 46.8% (111/237) and *A. phagocytophilum* prevalence was 8.4% (20/327). All serum samples tested negative for *E. canis, B. burgdorferi* and *L. infantum*. Prevalence of hookworm was 92.6% (185/204), *Trichuris vulpis* 6.9% (14/204), *Dipylidium caninum* 4.4% (9/204), *Toxocara canis* 3.4% (7/204) and *Capillaria* spp. 2.0% (4/204). Prevalence of *Giardia* spp. was 14.6% (7/48) while no *Cryptosporidium* was detected. Examination for external parasites was completed in 221 dogs. Fleas were found on 83.7% of the dogs (185/221), ticks on 42.1% (93/221) and lice on 8.1% (18/221). Identified ticks were *Rhipicephalus sanguineus*, with no *Ixodes* spp. found.

The results indicate a very high prevalence of hookworm, *D. immitis,* and external parasites in dogs in Samoa. This study provides valuable information on canine health and suggests dogs could play a role in the spread of some zoonoses in Samoa. Further studies are required to review the public health implications of this study.

**ABSTRACT ID-22**

**MOLECULAR EPIDEMIOLOGY AND ANTIFUNGAL SUSCEPTIBILITY AMONG CRYPTOCOCCUS ISOLATES FROM NORTH AMERICAN DOGS AND CATS..** LM Singer[1], W Meyer[2], GR Thompson[3], E Samitz[4], JE Sykes[5]. [1]Small Animal Clinical Sciences, Michigan State University College of Veterinary Medicine, East Lansing, MI, [2]Molecular Mycology Research Laboratory, The University of Sydney at Westmead Hospital, Westmead, NSW, Australia, [3]Department of Medical Microbiology, University of California, Davis, [4]Veterinary

Medical Teaching Hospital, University of California, Davis, [5]Department of Medicine & Epidemiology, University of California, Davis, CA

Cryptococcosis is caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. Eight molecular types have been described (VNI-VNIV for *C. neoformans* and VGI-VGIV for *C. gattii*). The objective of this study was to examine correlations between species, molecular type and antifungal drug susceptibility among canine and feline isolates from North America.

Cryptococcal isolates from dogs and cats from 2004 to 2011 were typed using restriction fragment polymorphism analysis and/or multilocus sequence typing. Susceptibilities to amphotericin B, fluconazole, itraconazole, 5-flucytosine, voriconazole, and posaconazole were determined using the Sensititre YeastOne® microdilution susceptibility test. The unpaired t-test and F-test were used to compare minimum inhibitory concentrations (MICs) between groups.

Fifteen canine and 27 feline isolates were examined. Most were from California (34); 3 were from Washington and Oregon. Molecular types were VGI (3), VGIIa (3), VGIIb (1), VGIIc (2), VGIII (16), VGIV (2), VNI (14) and VNII (1). All VGIII isolates were from cats in CA, AZ, and NV. The most common type in dogs was VNI (9 dogs). Flucytosine and amphotericin MICs were lower among *C. gattii* than *C. neoformans* isolates. For all drugs except itraconazole, *C. gattii* exhibited wider variation in MICs than *C. neoformans* isolates. VGIII MICs were not different to non-VGIII MICs. VGIII isolates exhibited wider variation in fluconazole and flucytosine MICs than non-VGIII isolates.

In conclusion, differences in drug susceptibility exist between *C. gattii* and *C. neoformans* isolates and molecular types from dogs and cats in North America. The clinical significance of these findings requires further investigation.

## ABSTRACT ID-23

## DEVELOPMENT OF AN INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF FELINE ANTIBODIES AGAINST *MYCOPLASMA FELIS*.

KT Wiggans, JR Hawley, MR Lappin. Department of Clinical Sciences, Colorado State University, Fort Collins, CO

*Mycoplasma* spp. are normal commensal organisms of the feline conjunctiva, genital tract and upper respiratory tract but also have been implicated in upper respiratory tract disease (URTD), lower respiratory tract disease, conjunctivitis and arthritis. Current detection methods for *Mycoplasma* include culture and polymerase chain reaction (PCR), both of which require specialized staff and facilities. Serology can be performed with minimal equipment and may be adapted for use at the point-of-care. The purpose of this study was to develop an indirect enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against *M. felis*.

A checkerboard titration was performed to determine the optimal IgG ELISA conditions using *M. felis* antigen produced from an isolate grown from a shelter cat with URTD. Sera from specific pathogen-free (SPF) cats (n = 6) prior to and after (10 days, 6 months and 12 months) exposure to *M. felis* and shelter cats (n = 52) with and without signs of URTD were tested in the ELISA. PCR assay for *M. felis* DNA was performed on conjunctival cells collected on swabs from the shelter cats. Potential for *M. felis* antibody cross-reactivity was tested by creating an IgG ELISA using *Ureaplasma* sp. antigen produced from an isolate grown from a shelter cat with URTD and used under the same ELISA conditions as the *M. felis* assay.

The precision of the optimized *M. felis* ELISA was estimated at approximately 5% by calculating the coefficients of variation for negative and positive absorbance values on 3 plates run on 3 separate days. When the same *M. felis* positive and negative sera were evaluated parallel in the *M. felis* and *Urealplasma* sp. IgG ELISAs, the percentage agreement was 92.3% suggesting a high degree of cross-reactivity between the antigen preparations. In the SPF cats, *M. felis* IgG absorbance values were significantly greater than pre-exposure absorbance values 10 days ($P < 0.001$) and 6 months ($P < 0.001$) post-exposure, but not 12 months ($P = 0.098$) suggesting that *M. felis* antibody titers may wane over time. Shelter cats with signs of URTD had significantly higher ($P < 0.001$) *M. felis* IgG absorbance values than healthy cats, but the percent agreement with PCR results was only 36.5%. Using the PCR assay results as the gold standard for infection, the specificity, sensitivity, positive predictive value and negative predictive value of the *M. felis* ELISA were 19.5%, 100%, 25% and 100%, respectively.

Cats experience a rapid and prolonged elevation in *M. felis* IgG antibodies following exposure to *M. felis*. Detection of *M. felis* IgG may prove to be a useful epidemiological tool for study of cats with URTD but appear to cross-react with other Mollicute species. While negative test results in cats with URTD can be used to exclude the diagnosis, positive test results do not accurately predict current infection.

## ABSTRACT ID-24

## DETECTION OF FELINE ANTIBODIES AGAINST A NOVEL *ANAPLASMA PHAGOCYTOPHILUM* PEPTIDE (AP-4) AFTER EXPOSURE TO WILD-CAUGHT ADULT *IXODES SCAPULARIS*.

MR Lappin[1], R Chandrashekar[2], B Stillman[2], J Liu[2]. [1]Department of Clinical Sciences, Colorado State University, Ft. Collins, CO, [2]IDEXX Laboratories, Portland ME

Cats that are exposed to *Anaplasma phagocytophilum* (AP) infected *Ixodes scapularis* ticks develop serum antibody responses to an immunodominant peptide (P44) used in a commercially available assay that is licensed for use with canine serum. The purpose of this study was to evaluate feline antibody responses to a novel AP peptide (AP-4) as a method for evaluating for early evidence of AP infection in cats.

Young, adult, mixed sex research cats (n = 4) were used in this pilot study with IACUC approval. Each of the cats was shown to be negative for antibodies against AP using a commercially available kit (SNAP® 4Dx®, IDEXX Laboratories) and negative for AP DNA in blood by use of a commercially available conventional PCR assay (Veterinary Diagnostic Laboratory, Colorado State University). Using the pre-inoculation samples, an ELISA for detection of antibodies against the AP-4 was optimized. Cats were infected with AP by exposure to wild-caught *Ixodes scapularis*. Blood for AP PCR assay and for serum separation for assessment for AP antibodies by SNAP® 4Dx® and AP-4 ELISA were collected prior to tick attachment and then on weeks 1 - 10 after tick attachment.

Each cat had AP DNA amplified from blood and each cat developed detectable AP antibodies in serum by both assays. Antibodies against AP were detected prior to those detected by the commercially available kit for 3 of 4 cats.

| Cat | *A. phagocytophilum* DNA | SNAP® 4Dx® | AP-4 ELISA |
|---|---|---|---|
| 1 | Weeks 2 - 5; 8 – 10 | Weeks 6 – 13 | Weeks 6 - 10 |
| 2 | Weeks 2 - 6, 8 - 10 | Weeks 5 – 13 | Weeks 2 – 10 |
| 3 | Weeks 2 – 5 | Weeks 5 – 13 | Weeks 3 – 10 |
| 4 | Weeks 2, 3, 6 | Weeks 5 – 13 | Weeks 3 - 10 |

The results suggest that antibodies against the AP-4 peptide may be useful for the early detection of AP exposure in cats and that further studies from samples collected from naturally exposed cats should be performed.

## ABSTRACT ID-25

## DETECTION OF CANINE SERUM ANTIBODIES AGAINST A NOVEL MUTANT PEPTIDE OF *ANAPLASMA PHAGOCYTOPHILUM*.

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*Anaplasma phagocytophilum* (AP) is the cause of granulocytic anaplasmosis in a variety of species including dogs. The purpose of this study was to validate a new automated system to detect

**Table 1.** % positive test results for 10 *I. scapularis* exposed dogs over time after tick placement.

| Assay/ Week | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PCR | 0 | 20 | 60 | 70 | 70 | 80 | 50 | 40 | 50 | 30 | 40 | 30 | 30 |
| AP Peptide | 0 | 0 | 10 | 10 | 70 | 90 | 90 | 90 | 90 | 90 | 70 | 70 | 60 |
| IFA | 0 | 0 | 20 | 30 | 60 | 90 | 90 | 90 | 100 | 90 | 90 | 90 | 90 |
| SNAP | 0 | 0 | 0 | 10 | 50 | 60 | 100 | 100 | 100 | 90 | 90 | 90 | 90 |

**Table 1.** Vaccine.

| | % Positive MAT Results – Week 3 | | | | | | | % Positive MAT Results – Week 7 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | B | C | G | H | I | P | Neg | B | C | G | H | I | P |
| 1 | 0 | 25 | 63 | 38 | 0 | 63 | 100 | 13 | 0 | 63 | 38 | 13 | 75 | 50 |
| 2 | 0 | 75 | 63 | 50 | 0 | 100 | 75 | 0 | 38 | 38 | 75 | 13 | 88 | 50 |
| 3 | 13 | 38 | 63 | 88 | 13 | 75 | 88 | 0 | 0 | 100 | 75 | 0 | 88 | 63 |
| 4 | 13 | 38 | 75 | 75 | 0 | 63 | 63 | 13 | 13 | 88 | 63 | 0 | 38 | 38 |

Vaccine 1 = Leptovax 4 (Boehringer-Ingelheim); Vaccine 2 = Nobivac Lepto4 (Intervet Schering-Plough); Vaccine 3 = Recombitek 4 (Merial); Vaccine 3 = Vanguard L4 (Pfizer Animal Health).

antibodies against AP using a novel mutant peptide of the organism.

Mixed sex beagles were inoculated with culture derived AP IV with serum and blood collected prior to inoculation, twice weekly for 4 weeks, and then weekly for an additional 4 weeks to provide samples for assay development. To assess the optimized assay using field-like conditions, adult *Ixodes scapularis* (average AP PCR positive rate = 15%) wild-caught in Rhode Island were allowed to feed on 18 mixed sex beagles for 7 days (13 female and 12 male per dog). Serum and blood in EDTA were collected prior to tick attachment and then weekly for 16 weeks. The AP mutant peptide was used as the capture reagent in a proprietary automated fluorescence system that is based on a silicon wafer. Titrations were performed to determine the optimal concentrations of the peptide, buffers, serum dilutions, and secondary antibody concentrations using samples from IV inoculated dogs. After the assay was optimized, sera from the dogs exposed to wild caught *I. scapularis* were assayed. The sera were also assayed for AP antibodies by IFA (Prototek Reference Laboratory, Chandler AZ) and a commercially available kit (SNAP®4DX; IDEXX Laboratories, Portland ME). In addition, a proprietary PCR assay (Antech Diagnostics, Lake Success, NY) was used to attempt to amplify AP DNA from blood from the tick exposed dogs.

The precision of the AP mutant peptide based assay based on determining the coefficient of variation of 20 positive samples assayed 4 times on different days was approximately 13%. Overall, 10 *I. scapularis* exposed dogs became PCR or IFA positive (Table 1). The time to first positive result for these 10 dogs varied by the assay but was statistically different amongst groups on week 3 when more samples were PCR positive than positive in any of the antibody assays.

Results of the 3 AP antibody tests were similar. Performance of AP PCR assays is indicated in dogs with suspected acute anaplasmosis if antibody assays are negative.

**ABSTRACT ID-26**

**VACCINE-ASSOCIATED *LEPTOSPIRA* ANTIBODY RESPONSES IN CLIENT-OWNED DOGS.** KT Wiggans[1], LER Martin[1], K Curtis[2], R Chandrashekar[2], MR Lappin[1]. [1]Colorado State University, Fort Collins, CO;, [2]IDEXX Laboratories, Portland ME

Antibody testing by microscopic agglutination test (MAT) is commonly used to aid in the diagnosis of canine leptospirosis. *Leptospira* vaccines are commonly administered to lessen illness associated with infection but induce antibodies that cannot be distinguished from those induced by natural infection. In addition, while multiple 4 serovar vaccines are now available in the United States, MAT responses after vaccination of client-owned dogs have not been widely reported. The purpose of this study was to determine the antibody responses of client-owned dogs vaccinated with 1 of 4 commercially-available 4-serovar *Leptospira* vaccines.

Healthy, client-owned dogs (n = 32) with no history of *Leptospira* vaccination for at least 1 year were enrolled. Dogs were randomly assigned to be administered 1 of 4 vaccines containing the *Canicola*, *Grippotyphosa*, *Icterohemorrhagiae*, and *Pomona* serovars with a booster vaccine 3 weeks later. Sera were collected prior to vaccination, week 3, and week 7. MAT titers against serovars *Bratislava* (B), *Canicola* (C), *Grippotyphosa* (G), *Hardjo* (H), *Icterohemorrhagiae* (I), and *Pomona* (P) were determined at Colorado State University.

One dog was positive for one serovar (1:200; I) prior to vaccination. Percentages of dogs developing a MAT titer of $\geq$ 1:100 varied between the weeks and amongst the vaccines (Table 1).

MAT titers $\geq$ 1:800 were detected for at least one serovar for 19 dogs (59.4%) on Week 3 and 20 dogs (62.5%) on Week 7. MAT titers as high as 1:6,400 were detected and 28 of 32 dogs developed at least one MAT titer $\geq$1:1,600. The most common serovars with MAT titers $\geq$ 1:800 after vaccination (Weeks 3 and 7 combined) varied amongst the vaccines, but were most commonly associated with C (31 samples), G (14 samples), P (13 samples), and I (10 samples).

*Leptospire* MAT titers after vaccination vary amongst client-owned dogs, the weeks after vaccination, and the vaccine product. MAT titers $\geq$ 1:800 will not always correlate to the presence of clinical leptospirosis if vaccines were recently administered.

**ABSTRACT ID-27**

**FELINE LEPTOSPIROSIS: A SEROLOGIC AND URINARY PCR SURVEY IN HEALTHY CATS AND IN CATS WITH KIDNEY DISEASE.** J Rodriguez, M-C Blais, C Lapointe, L Carioto, J Harel. Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada

Leptospirosis is a globally widespread zoonosis, with prevalence in cats varying from 8.8% to 35% depending on geographical localization. Although serologic evidence of feline exposure exists, clinical disease is rarely reported.

This study aimed to compare seropositivity and urinary PCR status for *Leptospira spp*. between healthy (H) and cats with kid-

ney disease (KD; acute and chronic KD IRIS stages II, III and IV).

Cats (n = 240) enrolled from January 2010 to December 2011 were aged-matched into two groups: H (n = 121) and KD (n = 119). A complete blood count, biochemistry profile, urinalysis, *Leptospira spp*. serology (titers $\geq$ 1:100 considered positive) and PCR were performed in all cats.

Seropositivity for *Leptospira spp*. was not statistically different between groups: 7.5% (n = 9) and 13.2% (n = 15) in the H and KD, respectively (p = 0.20). Serovars implicated were *L. pomona* (n = 14), *L. bratislava* (n = 9) *and L. grippotyphosa* (n = 1), with titers significantly higher for *L. pomona* (p = 0.04). Of the 24 seropositive cats, only one (H) was PCR positive. Similarly, no statistical difference was found in the PCR status between groups (H = 1, KD = 5; (p = 0.11)).

Sex did not influence serologic or PCR status. Seropositivity was greater in older cats ($\geq$ 10 years; p = 0.008), during summer (p = 0.005), and was more prevalent in healthy outdoor cats (p = 0.01) living in urban areas (p = 0.01) and in known hunters (p = 0.007).

No significant difference was found in the serologic and PCR leptospirosis status between H and KD cats, suggesting that they are good sentinels for leptospirosis monitoring and that renal colonization occurs, thereby creating a renal carrier.

## ABSTRACT ID-28

## ISOLATION AND CHARACTERIZATION OF *LEPTOSPIRA INTERROGANS* SEROVAR COPENHAGENI AND SEROVAR CANICOLA FROM DOGS WITH LEPTOSPIROSIS.

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*Leptospira interrogans* serovar icterohaemorrhagiae and serovar canicola are the most common serovars associated to leptospiral infection in dogs worldwide. In Brazil, serovar copenhageni is found more frequently as the etiological agent in both human and canine infection. Aiming to confirm the clinical diagnosis of leptospirosis, serum samples of 29 dogs presented with history and clinical syndromes associated to leptospiral infection (contact to rats, jaundice and/or acute renal failure) were submitted to microagglutination test (MAT) for anti-leptospiral antibodies. Additionally, urine samples were obtained by cystocentesis and cultured in Fletcher medium at 28-30°C for up to two months, in an attempt to isolate the leptospira involved. For antigenic and molecular characterization of the isolates, MAT with policlonal and monoclonal antibodies and VNTR-PCR were performed.

The serum MAT yielded negative results for six dogs; seven dogs reacted to one serovar and sixteen dogs reacted to two or more serovars, with antibody titers ranging from 100 to 3,200. At presentation, only eight (27.5 %) dogs had antibody titer $\geq$ 800. Paired serum samples obtained from 11 dogs revealed 4-fold increasing titers or seroconversion in five dogs, supporting a recent infection. Isolation attempts were successfully made in four dogs. Two of the isolates were inoculated in hamsters and re-isolated. Using specific anti-serum the isolates were identified as serovar copenhageni (sample A, titer 51,200) and canicola (samples B, C, titer 3,200). Sample A was identified as serogroup icterohaemorrhagiae when submitted to VNTR-PCR and the remaining samples as serovar canicola. Both serovar copenhageni and serovar icterohaemorrhagiae belong to the same serogroup icterohaemorrhagiae, and share the same molecular characteristics, therefore they could not be identified based on VNTR-PCR. The definitive identification was made by cross-reaction with specific monoclonal antibodies for each serovar (F70 C24, F70 C14-10, F12 C3-11 and F89 C12), which allowed the differentiation of both representatives of serogroup icterohaemorrhagiae. The isolate A showed immunological identity to serovar copenhageni (standard strain LI 130, FIOCRUZ – Bahia). Because of the poor ability of MAT to predict the serogroup or serovar of leptospira involved, the identification of infecting serovar should be based on the isolation of leptospiras from urine of dogs with clinical suspicion of leptospirosis.

## ABSTRACT ID-29

## NON-RIBOSOMAL GENE PHYLOGENY OF HEMOPLASMAS AND OTHER *MYCOPLASMA* SPECIES.

EN Barker, CA Hicks, IR Peters, CR Helps, S Tasker. School of Veterinary Sciences, University of Bristol, Langford, UK

Hemoplasmas are blood-borne bacteria that have been detected in many mammalian species, including cats and dogs. Previous phylogenetic analyses of the hemoplasmas have been based on 16S rDNA and ribonuclease P ribosomal gene (*rnpB*) data, or limited to the heat shock protein 70 gene (*dnaK*) of a couple of hemoplasma species. The aim of this study was to amplify and sequence fragments of the glyceraldehyde-3-phosphate dehydrogenase gene (*gapA*) and *dnaK* of a large number of hemoplasmas and *Mycoplasma fastidiosum* to use in phylogeny for the first time, and compare this to previously reported phylogeny.

DNA was purified from hemoplasma-positive blood and a freeze-dried ampoule of type-specific *M. fastidiosum*. *GapA* and *dnaK* sequences for available hemoplasmas, and selected other *Mycoplasma* species, were obtained from GenBank. Primers were designed to regions of hemoplasma *gapA* and *dnaK* using Primer3 and were used in polymerase chain reaction assays on the purified DNA to amplify gene fragments, which then underwent sequencing. Sequences were aligned using ClustalW run in MacVector v12. Phylogenetic trees were constructed for both *gapA* and *dnaK*.

*GapA* fragments (length range; 551-902bp) from 7 hemoplasma species (*Mycoplasma haemocanis*, *Mycoplasma haemomuris*, '*Candidatus* Mycoplasma haemohominis', *Mycoplasma coccoides*, '*Candidatus* Mycoplasma haemolamae', '*Candidatus* Mycoplasma haematoparvum' and '*Candidatus* Mycoplasma kahaneii') and *M. fastidiosum* were obtained. *DnaK* fragments (length range; 908-1710bp) from 5 hemoplasma species (*M. haemocanis, M. haemomuris,* '*Ca.* M. haemolamae', '*Ca.* M. haemohominis', and '*Ca.* M. kahaneii') were obtained. *GapA* and *dnaK*-based phylogeny showed that all hemoplasma species resided within a single clade, and that the hemoplasmas were further subdivided into two distinct groups, the haemofelis and haemominutum groups, as found previously with 16S rDNA- and *rnpB*-based phylogeny. *Mycoplasma haemofelis* and *M. haemocanis*, present within a single clade on 16S rDNA-based phylogeny, were in separate clades on both *gapA*- and *dnaK*-based phylogeny, confirming the previous *rnpB*-based phylogeny. Additionally, the *gapA*-based phylogeny did not cluster the hemoplasmas with members of the *pneumoniae* group of *Mycoplasma* species, including *M. fastidiosum*, which agrees with the *rnpB* phylogeny but contrasts to 16S rDNA- and *dnaK*-based phylogeny.

These results support previous work documenting the position of hemoplasma species within a single clade distinct from other members of the genus *Mycoplasma*, but more work is necessary to establish the relationships between the hemoplasma clade and other members of the genus *Mycoplasma*. It also indicates that *gapA*- and *dnaK*-based phylogeny may be useful in resolving relationships between highly related hemoplasmas.

## ABSTRACT ID-30

## FAILURE TO AMPLIFY *BARTONELLA KOEHLERAE* DNA FROM BLOOD OF CATS AND THEIR FLEAS IN THE UNITED STATES.

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*Bartonella koehlerae* DNA has been amplified from approximately 1-3% of *Ctenocephalides felis* removed from cats in France, Taiwan, and Thailand. In previous studies of cats and

their fleas in the United States, a PCR assay utilizing primers targeting a portion of the 16S-23S rRNA intergenic region that results in a different amplicon size for each *Bartonella* spp. has been used. Recently, it has been shown that in this PCR assay, *B. koehlerae* and *B. henselae* DNA have the same amplicon size which may lead to misidentification of the species if genetic sequencing is not performed.

Total DNA previously extracted from cats (n = 48 cats) and their *C. felis* (n = 47 flea groups [1 – 12 fleas per group]) that were positive for DNA of *B. henselae*, *B. clarridgeiae*, or both in the 16S-23S intergenic region *Bartonella* spp. PCR assay were selected. The DNA had been stored at - 80°C until used in this study. A conventional PCR assay with primers designed to amplify only the DNA of *B. koehlerae* was optimized (sensitivity = 1.5-2 bacteria/μl DNA) and applied to the DNA extracts.
Forward primer 5' CTTCTAAAATATCGCTTCTAAAAATT GGCATGC 3'
Reverse primer 5' GCCTTTTTTGGTGACAAGCACTTTTC TTAAG 3'

Based on the 16S-23S intergenic region *Bartonella* spp. PCR assay results, DNA of *B. henselae* (26 cat samples; 6 flea groups), *B. clarridgeiae* (15 cat samples; 16 flea groups), or both (7 cat samples; 25 flea groups) were amplified from the samples. When the *B. koehlerae* specific PCR assay was applied to the sample set, DNA of the organism was not amplified from any of the samples.

While *B. koehlerae* DNA was not amplified from this sample set, the prevalence rates in other studies of *C. felis* was $\leq$ 3%. Thus, the negative findings could merely relate to the sample size. However, the results suggest that *B. henselae* and *B. clarridgeiae* are the most common *Bartonella* spp. of cats and *C. felis* in the United States. If the 16S-23S intergenic region *Bartonella* spp. PCR assay is used, amplicons consistent with *B. henselae*/*B. koehlerae* should be sequenced.

**ABSTRACT ID-31**
**A FLEA AND TICK COLLAR CONTAINING 10% IMIDACLOPRID AND 4.5% FLUMETHRIN PREVENTS FLEA TRANSMISSION OF *BARTONELLA HENSELAE* IN CATS FOR 8 MONTHS.** MR Lappin[1], D Stanneck[2]. [1]Colorado State University, Department of Clinical Sciences, Fort Collins, CO, Bayer Animal Health GmbH, Leverkusen, Germany.

*Bartonella henselae* is transmitted amongst cats by *Ctenocephalides felis* and is associated with multiple clinical syndromes in cats and people. In a previous study, monthly administration of 10% imidacloprid was shown to block transmission of *B. henselae* amongst cats experimentally exposed to infected *C. felis*. The purpose of this study was to determine whether application of a flea and tick collar containing 10% imidacloprid and 4.5% flumethrin would lessen *C. felis* transmission of *B. henselae* amongst cats over an 8 month study period.

Specific pathogen free cats (n = 19) were housed in three adjoining enclosures that were separated by mesh to allow *C. felis* to pass among groups yet prevent cats from contacting one another. One group of 4 cats was inoculated intravenously with *B. henselae* and after infection was confirmed in all cats based on positive polymerase chain reaction (PCR) assay results, the cats were housed in the middle enclosure. The *B. henselae* infected cat group was flanked by a group of 8 cats that had the collar placed and maintained for the duration of the study and a group of 7 cats that was not treated. *Ctenocephalides felis* (50 males and 50 females) raised in an insectary were placed on each of the 4 cats in the *B. henselae* infected group monthly for 7 applications and then every 2 weeks for 4 applications starting the day the collar was applied. Blood was collected from all cats weekly for *Bartonella* spp. PCR, serology and culture.

While side-effects associated with the collars were not noted, persistent fever necessitating enrofloxacin therapy occurred in one untreated cat. While *B. henselae* infection was ultimately confirmed in 4 of 7 of the untreated cats, none of the cats with collars became infected (P = 0.026).

In this setting, use of a collar containing 10% imidacloprid and 4.5% flumethrin was well tolerated and prevented *C. felis* transmission of *B. henselae* amongst cats.

**ABSTRACT ID-32**
**CLASSIFICATION OF *BORRELIA BURGDORFERI* INFECTION USING RESULTS OF 5 ANTIBODY TARGETS IN AN AUTOMATED SYSTEM.** S Moroff[1], I Sokolchik[1], T Woodring[1], C Woodruff, B Atkinson[1], M Lappin[2]. [1]Antech Diagnostics, Lake Success, NY, [2]Department of Clinical Sciences, Colorado State University, Ft. Collins, Colorado

The purpose of this study was to validate a proprietary automated fluorescence system (Accuplex4™) that allows for simultaneous detection of 5 distinct serum antibodies against Bb in canine serum and differentiation of natural infection from vaccination and acute from chronic infection.

The Bb markers used in the Accuplex4™ included OspA, OspC, OspF, P39 and SLP. Multiple titrations with sera from naturally exposed or vaccinated dogs were performed to determine the optimal concentrations of the markers, buffers, serum dilutions, and secondary antibody concentrations. After the assay was optimized, sera from 36 mixed sex, young adult beagles that were exposed to wild caught *I. scapularis* (average Bb PCR positive rate = 56%) before (18 dogs) or after vaccination (18 dogs; 6 dogs per vaccine group) with three vaccines (Merck Nobivac Lyme; Pfizer LymeVax; Merial Recombitek Lyme) were assayed. Sera was collected from vaccinated dogs on Weeks 0, 1, 2, 3, 4, 5, 6, 7, 8, 11, 17 and 24. Sera was collected weekly from all dogs from weeks 24 through week 46. Results of Bb western blot immunoassays were also used in the development of proprietary computer algorithms to classify Bb infections based on antibody responses to the 5 targets.

Novibac Lyme induced positive Osp A, Osp C, and SLP responses, LymeVax induced positive Osp A and SLP responses, and Recombitek Lyme induced only Osp A responses. Vaccine induced antibody responses could generally be detected 2 to 3 weeks after the first vaccination. Of 18 dogs exposed to *I. scapularis* prior to vaccination, 14 developed evidence of infection with first positive antibody assay results detected from 14 to 70 days after tick attachment. Antibodies against Osp F were only detected in unvaccinated dogs with chronic infections ($\geq$ 7 weeks. When compared to western blot immunoassay, the Accuplex4™ detected antibodies for the first time on the same sample date (9 dogs), 1 week earlier (4 dogs) and 1 week later (1 dog).

Computer algorithm based evaluation of antibody response to multiple targets provides classifications that can differentiate vaccinated dogs, those with acute infections, and those with chronic infections and correlates closely with results of Western blot immunoassay. However, antibody test results must be interpreted in concert with clinical findings to diagnose clinical canine borreliosis.

**ABSTRACT ID-33**
**FELINE HISTOPLASMOSIS: FLUCONAZOLE THERAPY AND IDENTIFICATION OF POTENTIAL RISK FACTORS.** JM Reinhart[1], KS KuKanich[1], T Jackson[2], KR Harkin[1]. [1]Kansas State University College of Veterinary Medicine, Manhattan, KS, [2]Pittsburgh Veterinary Specialty and Emergency Center, Pittsburgh, PA

Feline histoplasmosis is a systemic fungal infection traditionally treated with itraconazole, which can be cost prohibitive for some clients. Additionally, although the clinical disease in cats has been thoroughly documented, risk factors have yet to be explored. The objectives of this study were to compare the outcomes of cats with histoplasmosis treated with fluconazole to those treated with itraconazole and evaluate possible environmental risk factors for affected cats. Medical records from feline patients with confirmed histoplasmosis (n = 32) at Kansas State University were systematically reviewed, and follow-up was performed by owner telephone interview.

One cat treated with fluconazole (n = 17) required a treatment change as compared with five cats treated with itraconazole (n = 13). Two cats were not treated. Overall mortality rates of cats treated with fluconazole (29%) and itraconazole (38%) were not significantly different (p = 0.27). Rates of recrudescence were also not significantly different (p = 0.29) when comparing cats treated with fluconazole (24%) to those treated with itraconazole (31%). At the authors' institution, a one month supply of itraco-

nazole for a 5-kg cat would cost $212.85 compared to $18.60 for fluconazole. Fluconazole is a viable, cost-effective alternative therapy for the treatment of feline histoplasmosis.

Forty-one percent of cats (12/29) represented in the follow-up survey were housed strictly indoors. Commonly reported environmental risk factors included exposure to indoor potted plants (13/29) and unfinished basements (12/29). These findings differ from the traditionally held view that histoplasmosis is strictly a disease of outdoor cats. Thus, other environmental risk factors warrant critical investigation.

## ABSTRACT ID-34

**EVALUATION OF *ENTEROCOCCUS FAECIUM* SF68 SUPPLEMENTATION WITH METRONIDAZOLE FOR THE TREATMENT OF NON-SPECIFIC DIARRHEA IN DOGS HOUSED IN ANIMAL SHELTERS.** A Fenimore, L Groshong, V Scorza, MRLappin. Department of Clinical Sciences, Colorado State University, Fort Collins, Colorado

*Enterococcus faecium* strain SF68 (FortiFlora, Nestle Purina PetCare) supplementation was shown in a recent study of shelter cats to lessen the incidence of non-specific diarrhea of $\geq$ 2 days duration when compared to placebo treated controls. SF68 is known to be resistant to metronidazole which is commonly used to treat shelter dogs with diarrhea. The purpose of this study was to determine whether dogs with non-specific diarrhea administered SF68 with metronidazole would have better clinical outcomes than dogs administered metronidazole alone.

A physical examination and a standardized fecal score system (7 = watery puddles; 6 = texture but no shape; 5 = moist piles; 4 = moist log shape; 3 = normal) was applied to all samples daily by a person masked to the treatment groups. Stray dogs with diarrhea without vomiting, a fecal score of $\geq$ 4, interest in food, and no clinical findings suggesting a foreign body were included. All dogs were fed a standardized diet and were administered metronidazole USP at 25 mg/kg, PO, twice daily for 7 days. The treated dogs were randomized to be administered SF68 (treatment) or a placebo mixed with their food daily for 7 days. Feces collected prior to treatment were analyzed by fecal flotation as well as fluorescent antibody assay for *Giardia* cysts and *Cryptosporidium* spp. oocysts. Speed to improvement was defined as the first day the score dropped 2 points from Day 0 or a fecal score of 4 was reached and sustained for 2 days in a row.

A total of 48 dogs were entered into the study by the time of abstract submission. Of these dogs, 33 dogs (16 treatment; 17 placebo) completed the study. Overall, 50% of the treatment group and 29.4% of the placebo group had fecal scores < 3 by day 7 ($p > 0.05$). However, speed to improvement was faster ($p = 0.036$) for the treatment group (mean = 2.8 days) compared to the placebo group (mean = 4.4 days). Both dogs with *Giardia* in the treatment group had normal feces by Day 7 in contrast to the controls where 3 of 7 dogs with *Giardia* had normal feces by Day 7.

In these dogs, administration of SF68 resulted in a faster speed to improvement than administration of metronidazole alone. A larger number of *Giardia* infected dogs will be needed to determine if dual treatment is superior to metronidazole alone for the treatment of this syndrome.

## ABSTRACT ID-35

**PREVALENCE OF AGENTS ASSOCIATED WITH CANINE INFECTIOUS RESPIRATORY DISEASE SYNDROME IN CLIENT-OWNED DOGS AND DOGS HOUSED IN SHELTERS.** C Karsten[1], JR Hawley[1], C Luttenegger[2], MR Lappin[1]. [1]Department of Clinical Sciences, Colorado State University, Ft. Collins, Colorado; [2]IDEXX Laboratories, Sacramento CA

Canine infectious respiratory disease syndrome (CIRDS) is a common in dogs housed in high density housing such as boarding facilities, day care, and animal shelters. The primary agents associated with this syndrome are *Mycoplasma* spp. (Myco), *Bordetella bronchiseptica* (Bb), *Streptococcus equi* var. *zooepidemicus* (SZ), adenovirus 2 (A-2), canine distemper virus (CDV), canine herpesvirus (CHV), parainfluenza (Para), canine influenza virus (CIV), and respiratory coronavirus (RCV). The purpose of this study was to use commercially available diagnostics to evaluate the prevalence of these agents in three groups of dogs.

Community-sick dogs were animals presented to their veterinarians or boarding facility with clinical signs consistent with CIRDS; shelter-sick dogs were animals that developed clinical signs of CIRDS while in a large open admission animal shelter; and shelter-healthy dogs were animals without clinical signs in the same shelter. Nasal discharges or materials from the caudal pharynx were collected onto 2 swabs with the swab containing Amies media transported to Colorado State University for *Mycoplasma* spp. culture and species determination. The other swab was transported to IDEXX Laboratories for performance of a proprietary PCR panel that amplifies the DNA or RNA of the primary agents associated with CIRDS.

CIV RNA (all dogs), SZ DNA (21 dogs), and H1N1 RNA (human influenza; 42 dogs) were not amplified from any dog. The distribution of results for other agents is listed in Table 1.

Shelter-sick dogs were more likely than community-sick dogs to be positive for a *Mycoplasma* spp. ($p = 0.002$). Multiple *Mycoplasma* spp. were grown but none were *M. cynos*. Coinfections with multiple agents were detected more frequently in shelter-sick dogs than community-sick dogs ($p = 0.03$).

Currently available diagnostic assay results for dogs with suspected CIRDS do not always correlate with clinical signs at the time of testing. *Mycoplasma* spp. appear to be associated with CIRDS in some dogs, but can be grown or amplified from healthy and sick dogs and it is currently unclear which *Mycoplasma* spp. are most likely to be pathogenic.

**Table 1.** Prevalence rates (%).

| | Myco | Bb | A-2 | CDV | CHV | Para | RCV |
|---|---|---|---|---|---|---|---|
| Community Sick (n = 23) | 43.5 | 13 | 4.3 | 0 | 0 | 0 | 4.3 |
| Shelter Sick (n = 24) | 87.5 | 25 | 25 | 8.3 | 4.2 | 4.2 | 25 |
| Shelter Healthy (n = 10) | 60 | 10 | 30 | 0 | 0 | 20 | 20 |

## ABSTRACT ID-36

**COMPARISON OF ELISA, CONVENTIONAL PCR, AND QUANTITATIVE PCR FOR DETECTION OF CANINE PARVOVIRUS.** Y Nakayama, MR Lappin, JK Veir. Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO

A point-of-care antigen ELISA is currently the most commonly used diagnostic test for canine parvovirus (CPV-2). However, it has low sensitivity compared to PCR based assays. This study compares the results of ELISA, conventional PCR (cPCR) and quantitative real time PCR (qPCR) in detecting CPV-2 in whole blood and feces of dogs suspected of being infected with CPV-2.

A biased group of samples was obtained from 49 dogs exhibiting clinical signs consistent with CPV-2 infection. DNA was extracted from both fecal and EDTA blood samples for cPCR and qPCR analyses. CPV-2 virus in the feces was also detected by a commercially available antigen ELISA. For all samples, a segment of the viral DNA was amplified and sequenced for genotyping.

Of the 49 fecal samples, 26 tested positive with ELISA. All of these samples were determined by a previously published qPCR assay to have a viral load of $>10^5$ viral particles/mg feces, the limit of detection for this ELISA. Real time PCR of the matching blood for these ELISA positive fecal samples showed that a viral load of $>10^5$ virus/ul blood could be detected in most but

not all cases (23/26). Conventional PCR detected CPV in the blood and feces from all dogs with positive ELISA results.

Seventeen of the 23 fecal samples that tested negative with ELISA were determined to have a viral load as determined by qPCR less than the limit of detection of the ELISA assay. However, there were 6 ELISA negative fecal samples with viral loads as determined by qPCR to be well above the limit of detection for ELISA. When all samples were genotyped, and 2% of the samples were CPV-2a, 50% CPV-2b, and 48% CPV-2c and differences in genotypes did not account for the discrepancy between qPCR and ELISA results seen above. Also, there were 6 samples that had positive results with both ELISA and cPCR, that initially were not detected by qPCR. However, using different primers and probes targeting a more conserved region of the CPV genome resulted in a high viral count as expected.

Overall, this study confirms that qPCR and cPCR of fecal samples are the most sensitive of the modalities presented. These results also suggest that because CPV-2 is a rapidly evolving virus, compatibility of existing diagnostic tests with current clinical strains should be tested periodically.

**ABSTRACT ID-37**

**PARVOVIRUS DNA COPY NUMBERS IN THE BLOOD OF CATS WITH AND WITHOUT HISTOLOGICALLY CONFIRMED PANLEUKOPENIA.** KP Polak, MR Lappin, S Mcleland, JK Veir, JR Hawley. Department of Clinical Sciences, Colorado State University, Fort Collins, CO

Use of real time polymerase chain reaction (qPCR) for quantitative detection of FPV and CPV2 DNA in samples from cats (qPCR-PV) has been shown to be both sensitive and specific over a wide range of viral titers in cats with naturally occurring FPV. Parvoviruses induce a viremia and so can be amplified from the blood. The purpose of this study was to determine the range of PV copies/µl blood in cats with suspected FPV infection with and without histological evidence of infection.

Blood, jejunum and ileum samples were collected from 25 shelter cats with clinical signs consistent with FPV after humane euthanasia. The majority of cats were vaccinated with a parenteral modified live FPV, FHV-1, and FCV containing vaccine upon intake to the shelter, prior to development of clinical signs. Blood in EDTA was evaluated in a previously validated qPCR-PV. Tissues were evaluated histopathologically after being stained with hematoxylin and eosin. The tissues were examined by an individual blinded to clinical history and qPCR-PV results. A diagnosis of FPV was given if characteristic lesions were present such as lymphoid depletion, intranuclear inclusions, villus blunting and fusion, or crypt necrosis, loss and abscessation.

In the 5 cats with histologically confirmed FPV infection, 4 cats were positive in blood by qPCR-PV with the PV copies/µl ranging from 0 – 89,333 (mean = 22,929; SD = 33,078). In the 20 cats with no histological evidence of FPV infection, PV copies/µl were < 3,000 in all cats with a range of 0 – 2,947 (mean = 319; SD = 790). PV copies/µl were < 100 in 17 of 20 cats.

The use of qPCR-PV performed on blood of kittens suspected to have FPV should be evaluated further for clinical utility by comparing to other currently available assays.

**ABSTRACT ID-38**

**TOXIN QUANTIFICATION OF *CLOSTRIDIUM PERFRINGENS* IS A PREDICTOR FOR DIARRHEA IN DOGS AND CATS.** CM Leutenegger[1], SL Marks[2], J Robertson[1]. [1]*IDEXX Laboratories, Inc., Sacramento CA*, [2]School of Veterinary Medicine, University of California, Davis

Toxigenic *Clostridium perfringens* strains are believed to be important contributors to gastrointestinal disease, and acute hemorrhagic diarrhea has been well documented in association with infection. Two toxins, the *C. perfringens* α-toxin and *C. perfringens* enterotoxin (CPE) are most frequently detected in dogs and cats, although questions have been raised regarding their toxigenic potential when detected in healthy animals. The objective of this work was to evaluate the relationship of toxin quantification with disease severity in diarrheic and apparently healthy dogs.

A total of 181 healthy and diarrheic dogs and cats were selected based on fecal scoring and analysis of clinical history. Toxin DNA and RNA quantification and toxin production were measured with commercial real-time PCR tests (IDEXX Laboratories, Inc.) and a commercially available enterotoxin ELISA (TechLab, Inc).

181 fecal specimens were evaluated, including, 48 from healthy dogs, 39 from healthy cats and 46 from diarrheic and 48 from diarrheic cats, respectively. When using a cutoff value determined by real-time PCR for toxin gene DNA quantification, the α-toxin gene was found to be significantly associated with diarrhea: the α-toxin gene was detected in 47.8% of diarrheic dogs vs. 4.2% of healthy dogs ($OR = 25.2$, $p < 0.0001$), whereas the α-toxin gene was detected in 37.5% of diarrheic cats vs. 12.8% of healthy cats (OR 4.08, $p = 0.01$). CPE was detected via ELISA in 19.6% of diarrheic dogs vs 2.1% of healthy dogs ($OR = 11.43$, $p = 0.007$), whereas CPE was detected in 8.3% of diarrheic cats vs. 0% of healthy cats ($OR = 7.99$, $p = 0.1$). Quantification of DNA from both toxins in diarrheic and healthy animals was significantly different in dogs and cats (Mann-Whitney test): α-toxin dogs: $p < 0.001$; α-toxin cats: $p = 0.0098$; CPE dogs: $p = 0.02$; CPE cats: $p < 0.0001$. All fecal specimens positive for toxin DNA were also positive for toxin gene mRNA indicating active transcription of the gene. This indicates that both the α-toxin gene and *cpe* are actively transcribing and synthesizing toxin proteins in toxigenic *C. perfringens* strains. The Pearson correlation between PCR and ELISA indicated a weak positive r value of 0.59.

In conclusion, we found that quantitative toxin gene analysis by real-time PCR for the α-toxin and CPE correlates with presence of diarrhea and has the potential to be used as a diagnostic marker.

**ABSTRACT ID-39**

**CANINE INFLUENZA VIRUS H3N8: A 4 YEAR REVIEW OF SEASONAL PATTERNS, GEOGRAPHICAL FREQUENCY DIFFERENCES AND AGE DISTRIBUTION.** CM Leutenegger[1], M Estrada[1], RD Armstrong[2]. [1]*IDEXX Laboratories, Inc., Sacramento, CA*, [2]Merck Animal Health, Summit, NJ

Canine influenza virus (CIV) H3N8 has been circulating in the canine population of the United States since early 2000. Using results from samples submitted by veterinarians, we analyzed epidemiological aspects of CIV occurrence between 2008 and 2011 including patterns in seasonal distribution, geographic occurrence and age classes of positive dogs.

A total of 17,331 swab samples (conjunctival and pharyngeal) submitted by veterinarians were analyzed over the 4 year period using a panel of real-time PCR tests specific for nine respiratory pathogens including CIV. A total of 311 CIV positive results were recorded. The yearly CIV PCR test positive proportion was 2008 - 3.7%; 2009 - 1.2%; 2010 - 1.7%; 2011 - 1.4%. In general, there was a seasonal distribution of positive results with peak numbers during either the winter months (January through March), or during summer to early fall (July - October). In 2008, outbreaks occurred both during winter (14 cases from 1 state) and summer (58 cases from 7 states). In 2009, a large outbreak occurred between January and March (40 cases from 7 states) and a second one between August and October (19 cases in 8 states). In 2010 two outbreaks were detected: one between April and June (25 cases in 6 states) and September (6 cases in 1 state). In 2011 three separate outbreaks were observed with a total of 28 cases in 6 states.

The main geographic locations with elevated case numbers were : 2008 - Illinois and Colorado; 2009 - Colorado; 2010 - North Eastern states; 2011- North Eastern states, Texas and California.. CIV positive cases were grouped into 4 age classes: < 6 months, 7-18 months, 19-84 months, and > 84 months. The average age for H3N8 positive dogs was 40 months. Positive tests were detected in all age classes, with the 19-84 month group infected most frequently (36%). In contrast, canine distemper virus PCR positive tests in our laboratory show an average age

of 13 months with the youngest age class (< 6 months) most likely to test positive.

These results highlight the dynamic situation of canine influenza virus H3N8 infections in the US dog population. Canine influenza has a seasonal pattern, tends to occur as outbreaks within a geographical area, and adult dogs (19 – 84 months) are the main age class affected.

**ABSTRACT ID-40**

**THE EFFECTS OF MAROPITANT VERSUS ONDANSETRON ON THE CLINICAL RECOVERY OF DOGS WITH PARVOVIRAL GASTROENTERITIS.** J Lenberg, L Sullivan, P Boscan, T Hackett, D Twedt. Department of Clinical Sciences, Colorado State University, Fort Collins, CO

Dogs affected with parvoviral gastroenteritis often require parenteral antiemetic therapy to control vomiting, avoid dehydration and enhance return of voluntary appetite. Maropitant acts centrally at the neurokinin-1 receptor to inhibit vomiting and may also provide visceral analgesia. The efficacy of maropitant has not been tested in dogs with parvovirus, but given its strong antiemetic properties, maropitant may be superior in controlling clinical signs when compared to traditional antiemetics. We hypothesized that hospitalized dogs treated with maropitant would demonstrate an improved clinical recovery and shorter duration of hospitalization compared to dogs treated with ondansetron.

Twenty-two naturally infected dogs, not previously treated for their disease, were included in the study following a positive snap ELISA parvoviral test. All dogs were treated with intravenous fluids, cefoxitin, and enteral nutrition. Dogs were randomized to receive either maropitant (1 mg/kg IV q24h, n = 11) or ondansetron (0.5 mg/kg IV q8h, n = 11) as a primary antiemetic. Frequency of vomiting and visceral pain/nausea scoring were evaluated twice daily. Rescue analgesics and antiemetics were administered as dictated by specific pain and nausea criteria. Clinical severity scoring, body weight, and caloric intake were monitored daily.

When comparing maropitant and ondansetron groups, there was no statistical difference in: duration of hospitalization (3.36 $\pm$ 0.56 vs. 2.73 $\pm$ 0.38 days, p = 0.36), frequency of rescue antiemetic use (3/11 vs. 5/11 dogs, p = 0.66), duration of vomiting (5 vs. 4 days, p = 0.65), days to voluntary appetite (2 vs. 1.5 days, p = 1.0), mean nausea score (27.9 $\pm$ 6.3 vs. 22.7 $\pm$ 5.9, p = 0.56), or mean CSU pain score (1.25 $\pm$ 0.18 vs. 1.10 $\pm$ 0.16, p = 0.87). Dogs treated with maropitant gained significantly (p = 0.01) more weight during hospitalization (0.10 $\pm$ 0.10 kg), whereas dogs treated with ondansetron lost weight (-0.43 $\pm$ 0.16 kg). Maropitant was administered intravenously in dogs $\geq$ 9 weeks of age, consecutively for up to 6 days, without any noted adverse effects.

In dogs with parvoviral gastroenteritis, maropitant appears to be equally as effective in controlling clinical signs when compared to ondansetron. Dogs treated with maropitant demonstrated improved ability to maintain body weight during hospitalization.

**ABSTRACT ID-42**

**IDENTIFICATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* OF ANIMAL ORIGIN USING BACTERIOPHAGE AMPLIFICATION AND A LATERAL-FLOW IMMUNOASSAY.** PS Morley[1], D Bolte[1], JD Rousseau[2], D Manna[3], B Dreiling[3], JS Weese[2]. [1]Colorado State University, Fort Collins, CO, [2]University of Guelph, Guelph, ON, [3]MicroPhage Inc., Longmont, CO

Methicillin-resistant *S. aureus* (MRSA) has emerged as an important health hazard for animals. Rapid laboratory confirmation is essential for appropriate clinical management and to allow appropriate control measures for minimizing nosocomial and zoonotic transmission risks. Recently, bacteriophage amplification has been used as the basis for a commercial test designed to rapidly identify MRSA in human clinical specimens. The purpose of this study was to evaluate the ability of a similar prototype test to identify animal-source MRSA and methicillin-susceptible (MS) *Staphylococcus aureus* (SA) and to evaluate the test's reactivity with relevant non-SA staphylococcal isolates.

Bacteriophage specific for SA were included in 2 broth media solutions, 1 that also contained cefoxitin to select for methicillin-resistant (MR) bacterial strains. A variety of genetically characterized staphylococcal isolates of animal origin were selected from an archival bank, and standardized concentrations were inoculated into the broth solutions. A lateral-flow immunoassay was used to detect phage that were amplified in overnight cultures of broth solutions.

Thirty-six MRSA isolates belonging to 7 major genetic types (USA 100, USA 200, USA 300, USA 500, USA 600, USA 700, and CC398) were evaluated. Thirty-two MRSA were correctly classified; 4/15 CC398 isolates were misclassified. Eleven of 15 MSSA isolates were correctly classified, as were 25 of 25 other non-SA staphylococcal isolates (including MR *S. pseudintermedius*, MS *S. pseudintermedius*, MR *S. hyicus*, MR *S. schleiferi*, and coagulase-negative *Staphylococcus* spp).

This prototype rapid assay correctly classified a high proportion of widely diverse MRSA, MSSA, and other non-SA staphylococcal strains of animal-origin. However the failure to detect some CC398 isolates might be problematic for some regions and animal species. Modification of the bacteriophage mixture may help overcome this misidentification and requires further study. This inexpensive, rapid method has promise for practical applications requiring identification of RSA colonization and infection in animals.

**ABSTRACT ID-43**

**PERFORMANCE OF THE NEW IN-CLINIC SNAP® 4DX® PLUS TEST FOR THE DETECTION OF *EHRLICHIA EWINGII* (GRANULOCYTIC EHRLICHIOSIS) AND *ANAPLASMA PLATYS* (THROMBOCYTOTROPIC ANAPLASMOSIS) ANTIBODIES IN DOGS.** BA Stillman[1], MJ Beall[1], M Monn[1], J Liu[1], B Thatcher[1], P Shields[1], B Andrews[2], SE Little[3], M Eberts[4], E Breitschwerdt[5], R Chandrashekar[1]. [1]IDEXX Laboratories, Westbrook, ME, [2]Town and Country Veterinary Clinic, Green Forest, AR, [3]Oklahoma State University, Stillwater, OK, [4]Lakeland Veterinary Hospital, Baxter, MN, [5]College of Veterinary Medicine, North Carolina State University, Raleigh, NC

The SNAP® 4Dx® Plus Test is an enzyme-linked immunosorbent assay (ELISA) for the simultaneous detection of canine heartworm antigen and antibodies to *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Ehrlichia ewingii* in canine serum, plasma or whole blood. The heartworm antigen assay is a sandwich assay utilizing a monoclonal and a polyclonal antibody as the capture and detection reagents, respectively. The SNAP 4Dx Plus Test uses the same reagents for *E. canis*, *Anaplasma*, and *B. burgdorferi* as in the current SNAP® 4Dx® Test, but an *E. ewingii* synthetic peptide derived from the major outer surface protein of *E. ewingii* has been added. The *Anaplasma* analyte detects antibodies to both *A. phagocytophilum* and *A. platys*. The SNAP 4Dx Plus Test was evaluated using a total of 1,195 samples. Test results were compared to confirmatory tests performed on each of the samples, as detailed in the Table below. To evaluate in-clinic performance, a field trial was conducted at four small animal veterinary practices and two academic veterinary medical centers, located in geographic regions where the SNAP 4Dx Plus is expected to have clinical utility. Results confirmed that SNAP 4Dx Plus can identify dogs with exposure to as many as five different tick-borne rickettsial organisms.

**Abstract ID-43: Table**

| Comparison Test | Sample Size SNAP 4Dx Plus / Reference Test +/+ | -/+ | +/- | -/- | Total | Sample Type | Relative Sensitivity and Specificity 95% Confidence limit |
|---|---|---|---|---|---|---|---|
| HTWM[1,2] | 94 | 1 | 2 | 269 | 366 | Serum/Plasma | Sen., 98.9% (95% CL 94.3%-99.8%) Spec., 99.3% (95% CL 97.3%-99.8%) |
| *A.phagocytophlium*[3] | 123 | 13 | 15 | 235 | 386 | Serum/Plasma | Sen., 90.4% (95% CL 84.3%-94.3%) Spec., 94.0% (95% CL 90.3%-96.3%) |
| *A. platys*[3] | 102 | 21 | 15 | 235 | 373 | Serum/Plasma | Sen., 82.9% (95% CL 75.3%-88.6%) Spec., 94.0% (95% CL 90.3%-96.3%) |
| *B. burgdorferi*[4] | 112 | 7 | 10 | 246 | 375 | Serum/Plasma | Sen., 94.1% (95% CL 88.4%-97.1%) Spec., 96.1% (95% CL 93.0%-97.9%) |
| *E. canis*[5] | 131 | 3 | 18 | 217 | 369 | Serum/Plasma | Sen., 97.8% (95% CL 93.6%-99.2%) Spec., 92.3% (95% CL 88.2%-95.1%) |
| *E. ewingii*[6] | 109 | 4 | 10 | 154 | 277 | Serum/Plasma | Sen., 96.5% (95% CL 91.3%-98.6%) Spec., 93.9% (95% CL 89.1%-96.7%) |

Reference [1]Necropsy and [2]petchek [3]A.phagocytophlium IFA [4]B. burgdorferi IFA [5]E. canis [6]E. ewingii ELISA

## ABSTRACT ID-44

**PREVALENCE OF SELECT VECTOR BORNE DISEASE AGENTS IN OWNED DOGS OF GHANA.** L Clarke[1], LR Ballweber[1], K Allen[2], S Little[2], MRLappin[1]. [1]Colorado State University, Fort Collins, CO and, [2]Oklahoma State University, Stillwater, OK

While many dogs in West Africa are seen in clinics for evaluation of clinical syndromes related to vector-borne diseases, such as anemia, weakness, and lethargy, the resources for definitive diagnosis are not available. The purpose of this study was to evaluate for evidence of vector borne infections in pet dogs in Ghana.

Ticks (n = 8), sera (n = 17), and blood samples in EDTA (n = 17) were collected from dogs evaluated in the Amakom Veterinary Clinic in Kumasi, Ghana between Dec 2010 and Jan 2011. The ticks were identified to the genus level by microscopy. Sera were evaluated for *Dirofilaria immitis* antigen and antibodies against *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Ehrlichia canis* (SNAP®4DX, IDEXX Laboratories, Portland ME). Conventional polymerase chain reaction assays designed to amplify the DNA of *Ehrlichia* spp./*Anaplasma* spp./*Neorickettsia* spp./*Wolbachia* spp., *Babesia* spp., *Rickettsia* spp., *Hepatozoon* spp., *Bartonella* spp. and the hemoplasmas were performed on DNA extracted from EDTA blood. Positive amplicons were sequenced.

All of the ticks were *Rhipicephalus* spp. Overall, 8 of 17 dogs (47%) were confirmed by PCR and genetic sequencing to harbor a vector borne agent (4 *Hepatozoon canis*; 2 *E. canis*; 1 *A platys*; 1 *Wolbachia*). In addition, 3 samples were positive for *A. phagocytophilum* antibodies, 2 samples were positive for *E. canis* antibodies and 1 sample was positive for *E. canis* antibodies and *D. immitis* antigen. Of the 3 dogs positive for *E. canis* antibodies, 2 were PCR positive and confirmed by sequencing to be *E. canis* and *Wolbachia*. One of the dogs that was PCR positive for *E. canis* and the *A. platys* PCR positive dog were negative for antibodies in the SNAP®4DX. The *Wolbachia* PCR positive dog was also positive for *D. immitis* antigen in serum. *Bartonella henselae* DNA was amplified from 2 ticks and *Hepatozoon canis* DNA was amplified from 1 tick.

Vector borne diseases are common in this part of Ghana and include *H. canis, E. canis*, *D. immitis*, and *A. platys*. *Bartonella henselae* is generally considered to be vectored by *Ctenocephalides felis* but has been amplified from both *Ixodes* and *Rhipicephalus* ticks. *Hepatozoon canis* is known to be carried by *Rhipicephalus* ticks and were the likely vectors in these dogs.

## ABSTRACT ID-45

**USE OF AN AUTOMATED SYSTEM FOR DETECTION OF CANINE SERUM ANTIBODIES AGAINST GLYCOPROTEIN GP36.** S Moroff[1], I Sokolchik[1], T Woodring[1], C Woodruff[1], B Atkinson[1], M Lappin[2]. [1]Antech Diagnostics, Lake Success, NY, [2]Department of Clinical Sciences, Colorado State University, Ft. Collins, Colorado

*Ehrlichia canis* (EC) is the most common cause of monocytotropic ehrlichiosis in dogs around the world. The purpose of this study was to validate a new automated fluorescence system (Accuplex4™) to detect antibodies against the EC immunodominant glycoprotein gp36.

Young adult, mixed sex beagles (n = 8) were inoculated with culture derived EC IV. Serum and blood samples (EDTA) were collected on Days 0, 3, 7, 10, 14, 17, 21, 28, 35, 42, 49, 63, 70, 77, and 84. Doxycycline was administered daily at 10 mg/kg, PO on Days 56 - 77. An assay to measure canine antibodies against gp36 in the Accuplex4™ was titrated with positive and negative control sera to determine the optimal concentrations of gp36, buffers, serum dilutions, and secondary antibody concentrations. After optimization, the sample set from the EC inoculated dogs was assayed. Sera were also assayed in an EC IFA (Prototek Reference Laboratory, Chandler AZ) and a commercially available kit (SNAP®4DX; IDEXX Laboratories, Portland ME). A complete blood cell count and a proprietary EC PCR assay (Antech Diagnostics, Lake Success, NY) were performed on each blood sample.

Results from the EC inoculated dogs varied by the assay with some statistical differences detected (Table 1). Onset of thrombocytopenia (< 170/cmm) occurred on Day 14 (4 dogs), Day 17 (1 dog) or Day 21 (3 dogs). On the day thrombocytopenia was first detected, PCR assay results (8 dogs) were positive more commonly than SNAP (1 dog) and IFA (2 dogs) but not more commonly than gp36 (4 dogs). Serum antibodies (all assays) but not EC DNA were detected after starting doxycycline.

**Abstract ID-45:**

**Table 1.** % positive results for 8 EC infected dogs.

| Assay/ Day | 0 | 3 | 7 | 10 | 14 | 17 | 21 | 28 | 35 | 42 | 49 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PCR | 0 | 0 | 0 | 0 | 75[a] | 87.5[a] | 100[a] | 100[a] | 100 | 87.5 | 87.5 |
| Low plat. | 0 | 0 | 0 | 0 | 50 | 62.5 | 100 | 100 | 87.5 | 87.5 | 87.5 |
| gp 36 | 0 | 0 | 0 | 0 | 12.5[b] | 87.5[a] | 100[a] | 100[a] | 100 | 100 | 100 |
| IFA | 0 | 0 | 0 | 0 | 0[b] | 50 | 75[a] | 100[a] | 100 | 100 | 100 |
| SNAP | 0 | 0 | 0 | 0 | 0[b] | 12.5[b] | 12.5[b] | 37.5[b] | 87.5 | 100 | 100 |

[a]is statistically different than [b]; plat = platelet.

EC DNA was amplified before seroconversion in any assay and correlated best with onset of thrombocytopenia. Antibodies against gp36 were detected within 3 days of PCR positive test results. Detection of EC antibodies in the Accuplex4™ assay occurs 3 to 21 days sooner than by SNAP and 7 to 14 sooner than IFA which likely reflects the cutoff value chosen for each assay.

# SMALL ANIMAL–NEPHROLOGY / UROLOGY

**ABSTRACT NU-1**
**PROTEIN-LOSING NEPHROPATHY ASSOCIATED WITH MUTATIONS IN NPHS1 (NEPHRIN) AND KIRREL2 (NEPH3/FILTRIN) IN SOFT-COATED WHEATEN TERRIERS.** MP Littman, CA Wiley, MG Raducha, PS Henthorn. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA

Soft-coated Wheaten Terriers are genetically predisposed to adult onset protein-losing nephropathy (average onset, 6.3 ± 2.0 years). A genome-wide association study, using the Illumina CanineHD BeadChip with 177 000 validated SNPs for genotyping, revealed a single statistically interesting region (p value 2.22 x $10^{-7}$) when comparing DNA samples from affected and geriatric ($\geq$14 years) unaffected Wheatens. Criteria for phenotypic selection included blood, urine, and histopathologic findings. Gene sequencing of candidate genes in the region revealed a single nucleotide change in each of two closely linked genes, NPHS1 and KIRREL2, which encode the slit diaphragm proteins nephrin and Neph3/filtrin, respectively. Mutations in nephrin and decreased expression of filtrin are associated with podocytopathy and protein-losing nephropathy in humans. The polymorphisms change a glycine to arginine in nephrin and a proline to arginine in a conserved proline-rich region in filtrin. These novel mutations are not described in other species, nor found in 753 dogs of 114 other breeds, except in 3 dogs, including 2 Airedales, 1 of which is doubly homozygous/affected and 1 is doubly heterozygous, and 1 Bloodhound, heterozygous for the filtrin mutation only. Risk for nephropathy is highest in dogs homozygous for the mutations (n = 186). Inheritance appears complex, with incomplete penetrance, variable expression, possibly additional genes and/or environmental triggers modifying the phenotype. This is the first canine inherited podocytopathy described.

**ABSTRACT NU-2**
**PRE-OPERATIVE PREDICTORS OF OUTCOME FOR CATS WITH SUCCESSFUL MANAGEMENT OF URETERAL OBSTRUCTIONS VIA URETERAL STENTING OR A SUBCUTANEOUS URETERAL BYPASS DEVICE.** C Horowitz, A Berent, C Weisse, C Langston. Animal Medical Center, New York, NY

Less-invasive alternatives to traditional surgical interventions for feline ureteral obstruction include stents and subcutaneous ureteral bypass (SUB). This study evaluated pre-operative parameters for prediction of hospitalization times, peri-operative survival, renal recovery, and long-term survival in cats with benign ureteral obstructions treated with a ureteral stent or SUB.

Medical records of 40 cats treated for ureteral obstruction(s) at the Animal Medical Center were reviewed. Pre-operative biochemical and imaging parameters were evaluated as predictors of length of hospitalization, survival to discharge, creatinine and IRIS stage at 3, 6 and 9 months post-procedure and overall survival.

There was a positive association between presenting creatinine (rho = 0.53, $p < 0.0008$), BUN (rho = 0.54, $p < 0.0006$), and potassium (rho = 0.40, $p < 0.0152$) with hospitalization time, while as presenting sodium decreased, the length of hospitalization increased (rho = -0.40, $p < 0.0217$). Cats with IRIS stages 1 and 2 at 3 months (median survival not reached vs. 272 days respectively, $p < 0.0014$) and 6 months (median survival not reached vs. 322 days respectively, $p < 0.0024$) post-procedure lived longer than those with higher IRIS stages. For every unit increase in presenting creatinine (HR = 1.11, $p < 0.0251$), BUN (HR = 1.01, $p < 0.0015$) and potassium (HR = 1.43, $p < 0.0476$), there was a higher hazard of dying.

Preoperative elevations in creatinine, BUN and potassium may be prognostic for longer hospitalization and decreased overall survival. Future studies should be performed to further assess these findings.

**ABSTRACT NU-3**
**EVALUATION OF WHITE COAT HYPERTENSION IN DOGS AND CATS USING THE PETMAP OSCILLOMETRIC BLOOD PRESSURE MONITOR.** SA Stewart, WW Muir, CE Langston. Animal Medical Center, New York City, NY

White coat hypertension is hypertension in hospital that normalizes at home. It is believed to result from stress-associated pressor release, and is documented in people, cats and dogs. If its magnitude is severe enough, patients may be erroneously diagnosed with true hypertension. The purpose of this study was to use the indirect oscillometric petMAP device to compare blood pressures in hospital with those measured at home by owners, to determine if we can differentiate between white coat and true hypertension. We performed a prospective observational study involving 55 client-owned animals (39 dogs and 16 cats) presented over a 15 month period. We collected a minimum of 6 systolic, diastolic and MAP measurements from each patient in hospital and then at home, and compared results using a paired 2-tailed t-test. We also compared the effect of temperament, age, gender, weight, disease status, administration of antihypertensives and limb used on blood pressure using non-paired 2-tailed t-tests and one-way ANOVA. There was a statistically significant difference between at home and in hospital readings for all parameters (dog systolic: 27 mmHg difference, $p < 0.0001$, diastolic: 14mmHg, $p = 0.0006$, MAP: 17mmHg, $p = 0.0005$; cat systolic: 20mmHg, $p = 0.015$, MAP: 14mmHg, $p = 0.03$) except cat diastolic (10mmHg, $p = 0.13$). Statistically significant differences were also found for limb used in hospital (37mmHg systolic higher in hindlimbs versus forelimbs, $p = 0.0009$, 16mmHg diastolic, $p = 0.02$, 26mmHg MAP, $p = 0.001$) and dog age for diastolic (13mmHg, $p = 0.025$) and MAP (17mmHg, $p = 0.014$) readings in hospital. We concluded that white coat hypertension exists and can be successfully documented.

**ABSTRACT NU-4**
**THE EFFECT OF BODY POSITION ON INDIRECT SYSTOLIC BLOOD PRESSURE MEASUREMENT IN DOGS.** DA Weinstein, ME Mackalonis, RS Hess. Department of Clinical Studies-Philadelphia, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

The effect of body position on indirect systolic blood pressure (BP) measurement in dogs is unknown. The objectives of this study were to determine whether there is a difference in systolic BP measurement by Doppler ultrasonography in sitting compared to recumbent body position and to determine the degree of BP measurement variability in each of these body positions.

A prospective, randomized, crossover study was performed. Systolic BP was measured in 51 healthy or sick dogs in sitting and laterally recumbent positions using Doppler ultrasonography. The position of the first set of BP measurements was determined randomly for each dog by a coin-flip. The first of seven consecutive measurements in each position was discarded from the analysis.

Mean systolic BP was significantly higher in the sitting (172.1 ± 33.3 mm Hg) compared to the recumbent position (147.0 ± 24.6 mm Hg, $P < 0.0001$). Systolic BP was higher in the sitting versus recumbent position in 86% of dogs (44/51). The mean difference in systolic BP measured in the two positions was 25.1 ± 28.5 mm Hg. Repeated BP measurements for each dog had a significantly ($P < 0.00006$) higher reproducibility in the recumbent position than in the sitting position.

BP measurements in dogs are significantly affected by body position and are higher in the sitting position than in a laterally recumbent position, for most dogs. The laterally recumbent body position is preferred for BP measurements because measurements in this position are less variable than in the sitting position.

**ABSTRACT NU-5**
**FEEDING A RENAL DIET IS ASSOCIATED WITH A REDUCTION IN PLASMA FIBROBLAST GROWTH FACTOR 23 (FGF-23) IN CATS WITH STABLE CHRONIC KIDNEY DISEASE (CKD).** RF Geddes, J Elliott, HM Syme. Royal Veterinary College, London, UK

FGF-23 is a phosphaturic hormone, which increases as renal function declines and is further elevated in hyperphosphatemic cats with azotemic CKD. FGF-23 concentrations decrease with dietary phosphate restriction in humans and rodents. The aim of this study was to investigate the effect of dietary phosphate restriction on plasma FGF-23 concentration in cats with stable azotemic CKD.

Azotemic (plasma creatinine concentration >2.0 mg/dl and USG <1.035) geriatric (> 9 years) cats seen at two London first opinion practices since August 2000 were identified. Cats offered a commercially available protein and phosphate restricted diet (Royal Canin Renal or Renal Special) were included in the study if they had blood samples taken on both the day they started diet (baseline) and 28-56 days later (on diet). Cats were excluded from the study if plasma creatinine concentration changed >25% during the study period, if the cat refused to eat the renal diet or if they had evidence of concurrent disease (with the exception of systemic hypertension controlled with medication). Cats were classified as hyper- or normophosphatemic based on the IRIS targets for plasma inorganic phosphorus concentration (iP): stage 2 target iP $\leq$4.5 mg/dl, stage 3 target iP $\leq$5 mg/dl, stage 4 target iP $\leq$6 mg/dl. Plasma FGF-23 concentrations were measured using a previously validated human intact FGF-23 ELISA. Comparisons between visits were made using the Wilcoxon signed rank test, with significance determined at $P<0.05$. Results are reported as median [25$^{th}$, 75$^{th}$ percentiles].

In hyperphosphatemic cats (n=16) eating renal diet was associated with a significant decrease in plasma concentrations of iP (from 5.9 [4.8, 7.4] to 4.1 [3.8, 4.5] mg/dl, $P<0.001$) and FGF-23 (from 1316.5 [491.9, 4227.6] to 537.7 [388.8, 1539.2] pg/ml, $P=0.008$), but not plasma creatinine concentration (2.7 [2.3, 3.2] vs. 2.7 [2.1, 2.9] mg/dl, $P=0.255$). In normophosphatemic cats (n=18) eating renal diet was associated with a significant decrease in plasma FGF-23 concentration (from 682.9 [352.9, 1610.4] to 561.3 [259.7, 1006.1] pg/ml, $P=0.006$), but not plasma concentrations of iP (3.5 [2.9, 4.0] vs. 3.3 [3.0, 3.7] mg/dl, $P=0.478$) or creatinine (2.6 [2.3, 3.0] vs. 2.4 [2.1, 2.9] mg/dl, $P=0.102$).

In conclusion, the feeding of a renal diet is associated with a reduction in plasma FGF-23 concentration in hyper- and normophosphatemic cats with stable azotemic CKD. This suggests that dietary phosphate and protein restriction has effects on phosphate homeostasis, even when plasma iP concentration does not significantly change.

**ABSTRACT NU-6**
**ACUTE KIDNEY INJURY IN DOGS HOSPITALIZED IN THE INTENSIVE CARE UNIT: A PROSPECTIVE STUDY (INTERIM ANALYSIS).** A Eatroff, C Langston. The Animal Medical Center, New York, NY

This report details an interim analysis of a study with the following objectives: determine the incidence of acute kidney injury (AKI) in the canine intensive care unit (ICU); identify risk factors for AKI in the ICU; and determine if AKI is associated with length of ICU and hospital stay, and hospital survival. Dogs were eligible for inclusion if the ICU stay exceeded 24 hours. Dogs were excluded if they weighed less than 2.5 kg or were diagnosed with AKI on admission. Serum creatinine concentrations were measured every 24 hours. Serial changes in creatinine were evaluated by the Risk, Injury, Failure, Loss of function, End-stage renal disease (RIFLE) and the Acute Kidney Injury Network (AKIN) schemes, and a newly proposed scheme (Cowgill). One hundred and nine dogs were included. All classification schemes reported similar incidences of AKI (9%, 10%, and 9%, respectively). Those receiving fluoroquinolones were more likely to develop AKI (odds ratio [OR], 4.4; 95% confidence interval [CI], 1.2 to 15.8) by cumulative failure type (AKIN, RIFLE, or Cowgill's scheme) and more likely to develop AKI (OR, 4.4; 95% CI, 1.1 to 17.9) by RIFLE. Those receiving diuretics were more likely to develop AKI (OR 4.6; 95% CI, 1.2 to 17.1) by cumulative failure type, more likely to develop AKI (OR 5.6; 95% CI, 1.4 to 21.7) by AKIN, and more likely to develop AKI (OR, 5.9; 95% CI, 1.4 to 22.9) by Cowgill's scheme. There was no association between AKI and lengths of ICU and hospital stays, or survival.

**ABSTRACT NU-7**
**LONG-TERM RENAL OUTCOME OF DOGS WITH ACUTE KIDNEY INJURY.** I Kis[1,2], A Schweighauser[1], T Francey[1]. [1]Vetsuisse Faculty University of Berne, Switzerland, [2]University of Zagreb, Croatia

Most studies on acute kidney injury (AKI) state only short-term survival data while long-term follow-up is very sparsely documented. All dogs diagnosed with AKI in 2007-2009 at the University of Berne were included in this study and grouped according to the underlying etiology as leptospirosis (L) and AKI from other causes (NL). Short- and long-term survival was determined 10d and >6m after discharge, respectively. Renal outcome of the survivors was re-assessed prospectively, including clinical and laboratory evaluation.

Of the 121 dogs diagnosed with AKI during the study period (89L, 32NL), 69 dogs were treated with hemodialysis (49L, 20NL). Median peak creatinine during hospitalisation was 739 µmol/l (IQR 491-1053). 63 dogs survived short-term (48L (54%), 15NL (47%)) and 47 of them could be re-evaluated (38L, 9NL). L-survivors were diagnosed with normal renal function (n=18, 47%), chronic kidney disease (CKD) IRIS stages 1 (n=13), 2 (n=3), 3 (n=3), and 4 (n=1). NL-survivors were diagnosed with normal renal function (n=4, 44%), CKD stages 1 (n=1), 2 (n=2), and 3 (n=2). Development of CKD was neither associated with the etiology group ($P=0.87$) nor with the presenting or the peak creatinine concentrations ($P=0.42$ and 0.29), but with the requirement for hemodialysis ($P=0.02$) in a univariate logistic regression analysis.

In conclusion, the high prevalence of CKD in dogs recovered from AKI warrants close long-term monitoring of their kidney function.

**ABSTRACT NU-8**
**ACUTE AND CHRONIC EFFECTS OF TEPOXALIN ON RENAL FUNCTION IN DOGS WITH CHRONIC KIDNEY DISEASE AND OSTEOARTHRITIS.** A Lomas[1], S Lyon[1], MW Sanderson[2], GF Grauer[1]. [1]Departments of Clinical Sciences, Kansas State University, Manhattan, KS, [2]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS

Chronic kidney disease (CKD) and osteoarthritis (OA) are common in older dogs. We assessed effects of tepoxalin on renal function in dogs with OA and Stage 2 or 3 CKD (serum creatinine [SrCr] 1.6-3.5 mg/dl). Inclusion criteria were OA and stable CKD (assessed by SrCr, iohexol plasma clearance, urine protein/creatinine ratio, enzymuria, and systolic blood pressure at -14 and -7 days). Dogs then received tepoxalin (10 mg/kg PO once daily) for 28 days (acute phase, 16 dogs), and 6 months (chronic phase, 10 dogs). Rechecks were performed weekly (acute phase) and then at 1, 3, and 6 months (chronic phase). Three owners elected not to continue beyond week 4.

Adverse events that resulted in withdrawal included: increased SrCr in one dog (week 1), collapse in one dog (week 1), increased liver enzymes in one dog (week 4), and vomiting, hematochezia, and GI ulceration in three dogs (weeks 8, 24, and 26, respectively). Renal function was not affected in the latter 5 dogs. Treatment discontinuation resulted in stabilized renal function in the first dog and resolution of 4 of the 5 adverse events. This rate of adverse events is similar to those listed for dogs with normal renal function.

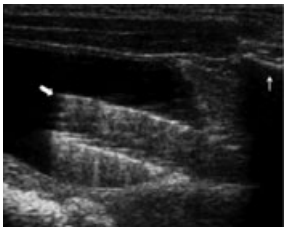
Renal function parameters were analyzed using repeated measures ANOVA. There was no difference over time for any parameter in dogs that were not withdrawn for adverse events. Results suggest that with close monitoring, tepoxalin may be used in dogs with OA and stable Stage 2 or 3 CKD.

**ABSTRACT NU-9**
**ULTRASOUND-GUIDED PLACEMENT OF URETHRAL STENTS IN DOGS.** T Archer, E Brinkman-Ferguson, A Dedeaux, B Geesaman, A Mackin. Mississippi State University College of Veterinary Medicine, Mississippi State, MS

Neoplasia, particularly transitional cell carcinoma and prostatic carcinoma, is a common cause of urethral obstruction in the dog. Stranguria is a common clinical sign, with partial or complete obstruction occurring in approximately 10% of these patients. One method for relieving malignant obstruction in dogs is the use of self-expanding metallic stents (SEMS). Placement of a urethral stent is a fast and effective means of rapidly achieving urethral patency with a reduced risk of complications compared to more aggressive surgical procedures. Stents are typically placed under the guidance of fluoroscopy, thereby limiting the availability of this technique to academic institutions and large referral practices.

The purpose of our study was to develop a technique for placement of SEMS under ultrasound guidance without fluoroscopy. Five canine cadavers (2M, 3F; wt 12-15 kg) were used. Positive contrast retrograde urethrocystography or vaginourethrocystography was performed prior to stent placement in males and females respectively to establish landmarks for assessment of stent location. Our goal was to place the cranial edge of the stent 2 cm cranial to the neck of the bladder. In males, a hydrophilic guide wire was inserted in the penile urethra and fed into the bladder, and the stent deployment device was passed over the wire. In females, a modified polypropylene urinary catheter was passed through the urethra into the bladder, and the deployment device was passed through the catheter. The bladder was expanded with a standardized fluid volume to facilitate ultrasonography. The cranial tip of the deployment device was identified on ultrasound and positioned 2 cm cranial to the bladder neck. The stent was then slowly deployed while the position of the cranial end of the stent was continuously monitored via ultrasound. Repeat radiographs were performed to determine the final position of the stent. In all 5dogs, the urethral stent was successfully placed within 0.5 cm of the target location.

Our study confirms that urethral stents can be successfully placed in normal dogs under ultrasound guidance without fluoroscopy. Our technique can be utilized in practices that do not have access to fluoroscopic equipment. Further investigation in tumor-bearing dogs is indicated.



Ultrasound image demonstrating a deployed urethral stent within the bladder. The thick arrow indicates the cranial edge of stent, and the thin arrow indicates the pubis.

**ABSTRACT NU-10**
**URINARY NGAL: A BIOMARKER FOR EARLY IDENTIFICATION OF ACUTE KIDNEY INJURY IN DOGS.** CA Palm[1], JL Westropp[1], B LeRoy[2], G Segev[3], LD Cowgill[1]. [1]UC Davis School of Veterinary Medicine, Davis, CA, [2]Abbott Laboratories , [3]Koret University

Despite advances in the treatment of acute kidney injury (AKI), the death rate among dogs remains high. A key factor contributing to AKI-associated fatalities is lack of sensitive tests allowing for early diagnosis. There is a growing interest in the development of biomarkers, which are natural substances that can be detected in urine or blood, and may allow for early AKI diagnosis. Neutrophil gelatinase-associated lipocalin (NGAL) is expressed in low concentrations in healthy dogs and humans, however, expression is markedly increased by compromised renal epithelium. Urinary NGAL (uNGAL) has been identified as one of the earliest and most robustly induced proteins in animal models of AKI. A canine specific ELISA kit for uNGAL has been developed for research purposes (BioPorto Diagnostics SA); pilot data shows uNGAL increases 7- to 200-fold in dogs with documented renal disease. Therefore, the purpose of this study was to evaluate the utility of uNGAL in a canine model of AKI.

Six dogs were administered gentamicin (10 mg/kg SC q 8hours) and uNGAL and serum creatinine were measured twice daily. Data available for two dogs revealed that uNGAL concentrations increased 7-fold above baseline by day 5 and 9, respectively, while serum creatinine remained within the reference range until days 14 and 16, respectively. When serum creatinine values increased by 50% in these two dogs, uNGAL had increased an average of 789-fold.

uNGAL may be a useful diagnostic tool for early detection of AKI in dogs and therefore may allow for improved patient outcomes.

**ABSTRACT NU-11**
**ACCURACY OF URICULT® VET PADDLES FOR THE DIAGNOSIS AND IDENTIFICATION OF BACTERIAL CYSTITIS IN DOGS AND CATS.** WL Ybarra, JE Sykes, YW Zingale, BA Byrne, PH Kass, JL Westropp. UC Davis School of Veterinary Medicine, Davis, CA

Quantitative urine culture is the gold standard for diagnosis of bacterial urinary tract infections, but this can be costly. The purpose of this study was to evaluate the accuracy of the Uricult® Vet system, an in-practice urine culture kit marketed for companion animal use.

In this prospective blinded study, urine specimens submitted to the UCD-VMTH microbiology laboratory for urinalysis and aerobic bacterial culture were selected by a veterinary technician for parallel analysis using the Uricult® Vet system. Paddles were inoculated by two investigators and incubated according to manufacturer's instructions. Each investigator's results were compared with each other and to those of the laboratory using McNemar's test.

A total of 207 urine specimens were analyzed, 64 of which yielded growth in the laboratory. The two investigators' results agreed for 190/207 (91.8%) paddle results. The mean sensitivity and specificity for identification of bacterial growth, regardless of the pathogen identified, was 92.4% and 98.9%, respectively. Investigator 1 correctly identified 52/64 (81.3%) and investigator 2 46/64 (71.9%) uropathogens. The mean sensitivity and specificity for identification of *Escherichia coli* was 88.7% and 98.0%; for *Enterococcus* spp. was 60.5% and 98.9%; and for *Staphylococcus* spp. was 61.6% and 99.0%, respectively.

The Uricult® Vet paddles appear to be a useful screening tool for identification of bacterial growth. However, inaccuracies occurred when paddles were used for organism identification, particularly when multiple uropathogens were present. When growth occurs, paddles or urine should be submitted to a commercial microbiology laboratory for identification and antimicrobial susceptibility testing.

**ABSTRACT NU-12**
**ASYMPTOMATIC BACTERIURIA *ESCHERICHIA COLI* STRAIN 83972 IN COMPETITION WITH EMERGING, HIGHLY VIRULENT MULTI-DRUG RESISTANT *ESCHERICHIA COLI* STRAINS IN CANINE URINE.** MF Thompson[1], JS Gibson[1], PC Mills[1], MA Schembri[2], JL Platell[1], DJ Trott[3]. [1]The University of Queensland, Gatton, QLD, [2]The University of Queensland, St Lucia, QLD, [3]The University of Adelaide, Roseworthy, SA

Deliberate colonization of susceptible dogs with the human asymptomatic bacteriuria *Escherichia coli* strain 83972 may repre-

sent a viable alternative for management of recurrent urinary tract infection (UTI). The strain outcompetes human uropathogenic *E. coli* (UPEC) in human urine *in vitro*, likely underpinning its success in prevention of recurrent UTI. We examined the growth of *E. coli* 83972 in competition with isolates representing three successful emerging multi-drug resistant (MDR) *E. coli* clonal groups in canine urine.

Mixed cultures were grown in pooled canine urine inoculated 1:1 with freshly grown pre-cultures of *E. coli* 83972 and one of three previously published successful MDR UPEC isolates cultured from canine UTIs (QUC07 [O75:ST1193]; QUC13 [ST131]; QUC18 [O15:K52:H1]). The cultures were grown aerobically at 37 $^{o}$C for 17 hours and viable counts were obtained. All experiments were performed in duplicate.

In two competition experiments (QUC07 vs. 83972 and QUC13 vs. 83972), there was no significant difference in mean viable counts at 17 hours. In the remaining competition experiment (QUC18 vs. 83972), *E. coli* 83972 was present in significantly lower numbers than the MDR *E. coli* strain after 17 hours ($P < 0.05$).

Given that the starting ratio in dogs following prophylactic bladder colonisation would favour *E. coli* 83972, it is feasible that it will outcompete MDR UPEC strains. Investigation of variables such as alternative bacterial concentrations, resistance, colicin production and virulence characteristics will enhance our understanding of the mechanisms by which *E. coli* 83972 grows in urine and its suitability for use in prophylactic treatment.

**ABSTRACT NU-13**
**GLOMERULAR FILTRATION RATE ESTIMATION ON THE BASIS OF PLASMA CLEARANCE OF INULIN IN DOGS.** M Nishida, M Uechi. Nihon University, Kanagawa, Japan

Accurate timed urine collections for the measurement of glomerular filtration rate (GFR) may be impractical in animals. The purpose of this study was to compare plasma clearance of inulin (P-CL) without urine collection with urinary clearance of inulin (U-Cin). The study included 23 healthy beagle dogs and 5 experimental dogs with renal failure. GFR was estimated on the basis of P-CL of inulin by using the trapezoidal method (P-CLTR) and 1-compartment method (P-CL1c) and on the basis of U-Cin. U-Cin was calculated following formula: U-Cin (ml·min-1) = urinary output of inulin (mg·min-1) /serum inulin concentration (mg·min-1). P-CL was calculated from the serum inulin concentration versus time data by using the basic formula: P-CL (ml·min-1) = dose of inulin (mg) /area under the curve (mg·ml-1·min-1). P-CLTR was calculated for 9 blood samples, and P-CL1c was calculated for 2 blood samples in each dog. Regression analysis was performed to establish correction formulas for predicting the U-Cin from the P-CL1c values. The U-Cin could be predicted from the P-CL1c values for 2 samples at 2 and 3 hours after inulin injection (P-CL1c 2,3) by using the formula U-Cin = -0.023 (P-CL1c 2,3)2 + 0.73 (P-CL1c 2,3) + 0.023 (R2 = 0.95). In conclusion, GFR was estimated on the basis of plasma inulin clearance with 2 blood samples at 2 and 3 hours after inulin injection.

**ABSTRACT NU-14**
**ESTIMATION OF GLOMERULAR FILTRATION RATE ON THE BASIS OF INULIN INFUSTION CLEARANCE IN DOGS WITH AND WITHOUT RENAL FAILURE.** M Nishida, M Uechi. Nihon University, Kanagawa, Japan

The gold standard method for measuring glomerular filtration rate (GFR) uses urinary inulin clearance (U-Cin). However, this method is not generally used because determining the exact urine volume in dogs is difficult. The aim of this study was to evaluate an inulin infusion-clearance (INF-Cin) method that does not require urine sampling for pharmacokinetic measurement of GFR during continuous inulin infusion in dogs. The study included 64 dogs with suspected renal dysfunction. GFR was simultaneously measured using U-Cin, urinary endogenous creatinine clearance (U-Ccr), and the INF-Cin method. U-Cin and U-Ccr was calculated by formula: U-Cin or U-Ccr (ml·min$^{-1}$) = urinary output of inulin or creatinine (mg·min$^{-1}$) /serum inulin or creatinine concentration (mg·min$^{-1}$). INF-Cin was calculated by formula: INF-Cin (ml·min$^{-1}$) = infusion rate of inulin (mg·min$^{-1}$) /serum inulin concentration (mg·min$^{-1}$). The mean (standard deviation) U-Cin, U-Ccr, and INF-Cin values were 2.6 (1.3), 2.8 (1.4), and 3.0 (1.1) ml·min$^{-1}$·kg$^{-1}$, respectively. A significant correlation was observed between U-Cin and INF-Cin ($R^2 = 0.91$, $P < 0.001$) and between U-Cin and U-Ccr ($R^2 = 0.82$, $P < 0.001$). However, the INF-Cin method overestimated the U-Cin in dogs with renal failure. In conclusion, these results indicate that the INF-Cin method can be used to measure GFR without urine collection in healthy dogs, whereas it may overestimate the GFR in dogs with renal failure.

**ABSTRACT NU-15**
**CLINICAL UTILITY OF PLASMA INULIN CLEARANCE AS A MEASURE OF GLOMERULAR FILTRATION RATE IN DOGS.** CP Sturgess[1], MD Dunning[2]. [1]Vet Freedom Ltd, Hampshire, UK, [2]University Nottingham, School of Veterinary Medicine and Science, Nottingham, UK

Glomerular filtration rate (GFR) is acknowledged to give a significantly more accurate assessment of renal function than standard biochemistry and urinalysis. Inulin clearance is well established as a 'gold standard' measure of GFR in humans. Further, inulin is commercially available, stable in plasma, requires a small sample volume, is inexpensive to assay and a canine reference range has been established.

A retrospective study of the clinical utility of using plasma clearance of inulin to measure GFR in dogs was performed investigating ease of use, quality of the clearance curve produced, potential side effects and clinical benefit.

Inulin clearance was performed on 45 dogs with 4 dogs having more than one test performed over time. A standard method was used giving 3000mg inulin/m$^2$ body surface area (Inutest 25% solution Fresenius Kabi) as an intravenous bolus via a catheter. Six timed blood samples were obtained over a 6 hour period (approximately 5-10, 20, 40, 100, 180 and 360 minutes). The exact time that the blood sample was acquired was recorded and used for data analysis. High pressure liquid chromatography was used to measure the inulin concentration in each sample by the same external laboratory (Alomed). GFR was calculated using commercial software (Inusoft - Laevosan).

Timed blood samples were obtained in all dogs (weight range 4-55kg, 17 breeds, 10-156 months of age). No side effects were observed following inulin injection, during the test period or over subsequent hospitalisation. The results obtained produced a good curve-fit in the majority of cases. Occasional aberrant results occurred that were unexplained and were discarded by the software. Calculated inulin clearances were from 12 to 140ml/min/m$^2$ [ref: 85-144].

The use of inulin to measure GFR was straightforward, reproducible and without observed side effects. It was felt to be of value to diagnose or rule-out early renal disease (IRIS stage I and II) in non-azotemic dogs with polyuria/polydipsia, to assess the extent of long-term renal damage following acute renal failure in patient's whose urea and creatinine had normalized and to monitor progression of cases to help decide whether further intervention e.g. biopsy was appropriate. The value in dogs with established IRIS stage III-IV renal disease was unclear.

**ABSTRACT NU-16**
**AST-120 ATTENUATES SERUM LEVELS OF THE UREMIC TOXIN, INDOXYL SULFATE, IN CATS WITH DECREASED RENAL MASS.** BH Schmidt[1], R Krebber[2], M Murphy[3], FJvan derStaay[4]. [1]Bayer Animal Health GmbH, Leverkusen, Germany, [2]Bayer CropScience AG, Monheim, Germany, [3]Charles River Laboratories Preclinical Services Ireland Ltd., Ballina, Ireland, [4]University of Utrecht, The Netherlands

Previous work has shown that there is an accumulation of the putative uremic toxin, 3-Indoxyl sulfate (IS), in serum of cats

with chronic kidney disease (CKD) similar to the situation in human CKD patients. Since IS is derived from bacterial tryptophan metabolism to indole in the intestines, the present study was undertaken to assess whether the potent activated carbon-based adsorbent, AST-120 (also known as KremezinTM drug substance or CovalzinTM, Kureha Corp., Tokyo, Japan) is able to reduce the systemic burden of IS in cats with reduced renal mass.

Two experiments were performed using the same colony of laboratory cats that had undergone a unilateral nephrectomy and ligation of branches of the renal artery supplying the remaining kidney. Both were approved by the local ethical committee. At the start of the first study, 12 cats were switched to a suitable moist diet in order to allow for mixing the test item into the feed. The animals were sequentially treated with 0 (control phase), 50, 100 and 200 mg AST-120 per kg body weight daily. Each dose was administered for a period of 14 days. This treatment series was completed with a 2-week wash-out period. The second study involved 11 of the cats which participated in the first experiment and consisted of three successive periods of 4 weeks each: no treatment, AST-120 100 mg/kg daily, and a final wash-out. In both experiments, serum samples were taken at study start and at the end of each dosing phase. The primary efficacy variable was serum IS. Secondary variables comprised serum levels of creatinine, urea, and inorganic phosphate. The cats remained on the same diet from the start of the first experiment to the end of the second one. The results were statistically analyzed by repeated measures ANOVA, followed by t-test. The level of statistical significance was $p < 0.05$.

Serum IS levels were significantly lower than baseline following administration of 100 and 200 mg AST-120 per kg bodyweight, but there was no difference between administration of these two doses. No difference from baseline could be observed for the dose of 50 mg/kg. All parameters except serum urea, which was reduced compared to baseline, were unaffected at 100 mg/kg. The second study confirmed the attenuation of serum IS levels by the dose of 100 mg/kg, while the reduction in serum urea levels was not confirmed. In both studies, the AST-120 induced changes were not abolished and persisted beyond the wash-out period. AST-120 was well tolerated by the study animals at all doses tested.

The results indicate that the dose of 100 mg AST per kg body weight is necessary and sufficient to mediate a significant and clinically relevant reduction in serum IS levels. Further studies are required to investigate the reason for the persistence of the beneficial effects upon discontinuation of treatment.

## ABSTRACT NU-17

**EVALUATION OF 3 STRUVITE-OXALATE PREVENTATIVE DIETS IN HEALTHY CATS.** T Gluhek, JW Bartges, A Callens, G Galyon, S Cox, CA Kirk. College of Veterinary Medicine, The University of Tennessee, Knoxville, TN

There are 3 feline "oxalate-struvite" preventative diets available. We hypothesized that (a) these 3 diets would induce lower urinary saturation estimated by relative supersaturation for struvite ($RSS_{struvite}$) and calcium oxalate ($RSS_{caox}$) than an adult maintenance diet.

Six healthy shorthair cats, 2 FS aged 3 yr, 2 FS aged 6 yr, and 2 MC aged 3 yr, were randomly assigned to consume 1 of 3 dry struvite-oxalate preventative diets (CD = Prescription Diet c/d MultiCare, SO = Royal Canin s/o, and UR = Purina UR St/Ox) or a maintenance adult food (M = Science Diet Maintenance) in a Latin-square design. Diets fed for 3 weeks and a 24-hour urine sample was collected. All cats consumed all 4 diets. Data were analyzed using ANOVA with significance of $p < 0.05$.

Differences were not found for body weight; 24-hour excretions of magnesium, oxalate, citrate, or creatinine; or $RSS_{struvite}$ and $RSS_{caox}$ between diets. Differences between diet groups were found for 24-hour urinary excretion of sodium, potassium, calcium, ammonia, phosphorous, chloride, and 24-hour urine volume.

Despite differences, estimated RSS were not different between CD, SO, UR, and M, although $RSS_{caox}$ tended to be highest on M and lowest on UR. Twenty-four hour urinary excretion of sodium and chloride and urine volume was greatest with SO and UR because these diets contain greater amount of sodium chloride than CD and M; 24-hour urinary excretion of calcium was lowest with CD. The 3 oxalate-struvite preventative diets tend to induce a lesser degree of urine saturation for calcium oxalate than the maintenance diet by differing dietary modifications.

## ABSTRACT NU-18

**EVALUATION OF CULTURE TECHNIQUES AND BACTERIAL CULTURES FROM CANINE AND FELINE UROLITHS.** L Perry, PH Kass, DL Johnson, R Shiraki, AL Ruby, JL Westropp. University of California Davis School of Veterinary Medicine, Davis, CA

Canine struvite uroliths can form due to an infection, while others can act as a nidus. Published techniques for culturing uroliths are variable; washing the uroliths four times and culturing the core has been reported. The objectives of this study was twofold:1) prospectively evaluate urolith culture protocols and 2) report bacterial isolates from uroliths and their association with signalment, stone type, and previous antimicrobial use.

For part 1, 50 urolith pairs were cultured by washing each stone either 1or 4 times and then culturing the core. For part 2, a retrospective search of isolates collected from uroliths submitted to our laboratory was recorded; the signalment, stone type, and previous antimicrobial use were reviewed. McNemar's Tests were performed on the prospective data. A multiple logistic regressions were used to analyze the retrospective data.

Regarding stone culture techniques, no differences in bacterial isolates from the urolith core were found. Washing the stone four times does not appear warranted. In part 2, we evaluated culture results from 520 canine and 168 feline uroliths. We found struvite-containing uroliths had an increased risk for positive culture

**Abstract NU-17:**

**Table.** Twenty-four hour excretions of analytes, urine volume, and relative supersaturation from 6 cats consuming 4 diets. Results with different superscript letters differ at $p < 0.05$.

| Analyte | Units | CD | SO | UR | M |
|---|---|---|---|---|---|
| Sodium | mEq/kg | $1.29 \pm 0.32^{a}$ | $5.64 \pm 1.23^{b}$ | $6.24 \pm 1.96^{b}$ | $1,32 \pm 0.30^{a}$ |
| Potassium | mEq/kg | $2.10 \pm 0.54^{ab}$ | $2.97 \pm 0,57^{ac}$ | $3.08 \pm 0.85^{c}$ | $1.69 \pm 0.19^{b}$ |
| Calcium | mg/kg | $0.18 \pm 0.07^{a}$ | $0.43 \pm 0.22^{b}$ | $0.34 \pm 0.13^{ab}$ | $0.29 \pm 0.10^{ab}$ |
| Ammonia | mM/kg | $0.17 \pm 0.15^{a}$ | $0.52 \pm 0.16^{b}$ | $0.55 \pm 0.28^{b}$ | $0.21 \pm 0.37^{a}$ |
| Phosphorous | mg/kg | $26.9 \pm 4.9^{a}$ | $27.0 \pm 5.6^{a}$ | $44.1 \pm 11.9^{b}$ | $27.4 \pm 3.5^{a}$ |
| Chloride | mEq/kg | $2.14 \pm 0.85^{a}$ | $6.13 \pm 1.36^{b}$ | $7.69 \pm 2.46^{b}$ | $1.85 \pm 0.32^{a}$ |
| Volume | ml/kg | $10.4 \pm 1.8^{a}$ | $17.5 \pm 2.5^{ac}$ | $19.2 \pm 6.5^{bc}$ | $11.7 \pm 4.5^{a}$ |
| $RSS_{coax}$ | | $1.48 \pm 0.76^{a}$ | 1.53 1.00 [a] | $1.15 \pm 0.79^{a}$ | $2.53 \pm 1.26^{a}$ |
| $RSS_{struvite}$ | | $0.041 \pm 0.059^{a}$ | $0.034 \pm 0.038^{a}$ | $0.089 \pm 0.089^{a}$ | $0.017 \pm 0.059^{a}$ |
| pH | | $6.09 \pm 0.17^{a}$ | $5.98 \pm 0.12^{a}$ | $6.14 \pm 0.29^{a}$ | $5.92 \pm 0.18^{a}$ |

results compared to non-struvite ($P < 0.0001$, OR 5.4, CI 3.5-8.4), as did uroliths from female dogs ($P < 0.0001$, OR 2.9, CI 41.9-4.3). Dogs < 7 years had a decreased risk for positive urolith culture ($P < 0.0001$, OR 0.4, CI 0.3-0.6). There was no significant difference between culture results and previous antimicrobial administration ($P = 0.4$), suggesting stone cultures may be warranted despite this. Analyzing urolith cultures may help guide patient management and urolith prevention.

**ABSTRACT NU-19**
**THE USE OF DARBEPOETIN ALFA IN CATS WITH CHRONIC KIDNEY DISEASE.** JE Markovich, MA Labato, EH Fiocchi, EA Rozanski. Tufts University Cummings School of Veterinary Medicine, North Grafton, MA

End stage chronic kidney disease (CKD) is often associated with the development of non-regenerative anemia due to decreased production of endogenous erythropoietin. Therapy with recombinant human erythropoietin is effective at raising the hematocrit, but is associated with the possible development of pure red cell aplasia (PRCA). Darbepoetin alfa is a newer synthetic human recombinant erythropoietin analog and has been suggested as less likely to cause the development of antibody-associated PRCA.

The purpose of this study was to describe the use of darbepoetin in cats with CKD in a prospective observational study. Cats with CKD IRIS stage II-IV, with a non-regenerative anemia of $\leq$ 23% were enrolled in the study. Darbepoetin at a dose of 1 mcg/kg/week was administered intravenously or subcutaneously until the patient PCV = 30%. Blood pressure, PCV/TS, and creatinine were monitored throughout the study. Descriptive statistics were used.

Seven cats were enrolled. The length of time to achieve a PCV $\geq$ 30% was a median of 47 days (range 7-52 days). No cats developed new anemia during therapy or other evidence of PRCA. Survival times from the initiation of darbepoetin therapy to death ranged from 42-296 days (mean 133 days; median 78 days). All cats were IRIS Stage III or IV throughout the study. At achievement of the target PCV of 30%, there was no significant increase in blood pressure in any cat from the start of the study, and in one cat the blood pressure normalized.

In this pilot study, 1 mcg/kg/week was effective in raising the hematocrit of cats with CKD without evidence of pure red cell aplasia. Further investigation of darbepoetin in cats is warranted.

**ABSTRACT NU-20**
**INTRAVESICAL GLYCOSAMINOGLYCANS AS A NOVEL THERAPY FOR FELINE IDIOPATHIC CYSTITIS – A PILOT STUDY.** A Bradley, MR Lappin. Department of Clinical Sciences, Colorado State University, Fort Collins, CO

Feline idiopathic cystitis (FIC) is a common, frustrating condition with few proven therapeutic options. Its recurrence rate is approximately 50% and FIC can result in urethral obstruction. One of the proposed causes of FIC is a defective glycosaminoglycan (GAG) layer lining the urinary bladder mucosa. Exogenous GAGs adhere to damaged bladder uroepithelium, and promising data exist for their use in human interstitial cystitis. The objectives of this prospective study were to evaluate the safety of intravesical GAGs while gathering pilot data for a larger study.

At the time of abstract submission, 13 cats presenting for urethral obstruction were enrolled. Previously treated cats, as well as cats with severe azotemia or hyperkalemia, urolithiasis, and/or uroabdomen were excluded. Cats were treated as similarly as possible (sedation or anesthesia for urinary catheter placement, fluid therapy, buprenorphine, phenoxybenzamine, canned urinary diet) and randomized to receive either intravesical GAGs (hyaluronic acid, chondroitin sulfate, glucosamine; A-CYST, Arthrodynamics Laboratories, Lexington, KY) or intravesical saline placebo at the time of catheter placement and again 12 and 24 hours later. Outcomes included urinalysis at day 0, 3, and 7, urine culture at day 0 and 7, standardized pain scoring, and incidence of repeat urethral obstruction.

There was no significant difference in presenting potassium or creatinine levels or in pain score between the treatment group (n = 6) and the placebo group (n = 7). The mean urine specific gravity of the treatment group was 1.030 versus 1.043 for the placebo group ($p = 0.05$), and the mean protein content of the treatment group was 2.8 versus 1.6 for the placebo group ($p = 0.03$). Three of the cats that received the placebo developed repeated urethral obstruction within one week of initial presentation with 2 of 3 cats re-enrolled in the treatment group. When these results were combined, 3 of 7 cats (42.9%) in the placebo group and 0 of 8 cats in the treatment group developed a repeat obstruction ($p = 0.08$). No adverse reactions to intravesical GAGs were observed. Three of the 12 post-catheter urine cultures were positive (one each of methicillin-resistant *Staphylococcus aureus*, *Streptococcus sp.*, and *Escherichia coli*). There was no difference in incidence of urinary tract infection between the treatment and placebo groups. Subjectively, urine sediment became more benign in all cats over time.

This GAG was previously shown to be safe administered IM and appears to be safe for instillation into the urinary bladders of cats with urethral obstruction. A lower percentage of treated cats developed a repeat urethral obstruction compared to the saline placebo, however, results were not significantly different, and so larger studies are indicated to further evaluate the efficacy of this novel therapeutic option for FIC.

# SMALL ANIMAL – NEUROLOGY

**ABSTRACT N-1**
**CEREBELLAR ABIOTROPHY IN FOUR RELATED POMERANIANS.** C Levine[1], J Evans[1], RC Longshore[2], SB Plummer[1], GC Johnson[3]. [1]Veterinary Neurological Center, Phoenix, AZ, [2]Gulf Coast Veterinary Specialists, Department of Neurology/Neurosurgery, Houston, TX, [3]University of Missouri, Veterinary Medical Diagnostic Laboratory, Columbia, MO

Cerebellar abiotrophy is the result of premature death or degeneration of related groups of neurons, usually Purkinje cells, in the cerebellar cortex, due to an inborn metabolic error resulting in their unexpected degeneration. This disease has been described in a number of the domesticated species, primarily the dog, but also in the cat, cattle, sheep, and horses. The disease can manifest as neonatal, juvenile, or adult onset forms and an autosomal recessive pattern of inheritance has been described in several species. The symptoms of the disease are characteristic of cerebellar dysfunction, such as ataxia, dysmetria, falling, intention or generalized tremors, and occasionally apparent blindness, though not all clinical signs may be manifested. Four purebred young adult Pomeranians with a history of slowly progressive signs of cerebellar ataxia such as hypermetria, falling over backwards, and visual deficits were examined. Diagnostic tests were unremarkable and empirical treatment did not alter the progression of signs. Necropsy was performed on 3 of the dogs and identified extensive loss of Purkinje neurons consistent with a diagnosis of cerebellar abiotrophy. Analysis of the pedigrees showed that these dogs were directly related and an autosomal recessive mode of inheritance was likely. Cerebellar abiotrophy has been best characterized in the dog, but has not been previously reported in the Pomeranian.

**ABSTRACT N-2**
**DOES CEREBROSPINAL FLUID CYTOLOGY PREDICT CENTRAL NERVOUS SYSTEM HISTOLOGY?** U Jeffery, N Jeffery. Iowa State University, Ames, IA

CSF analysis of cell count, cytology and total protein has been shown to provide diagnostically useful, but non-specific, information. However, CSF samples are often low volume, so tests must be prioritized. This study determined how frequently CSF cytology alone was able to predict: i) the precise histologic diagnosis;

or ii) a category of histologic diagnosis with a single treatment implication (e.g. immune mediated disease).

Medical record search identified 50 dogs and 7 cats that had a CSF cytology report and CNS post mortem histology determined within 14 days. Using standardized criteria, CSF cytology were classified as: i) 'non-diagnostic quality'; ii) 'no abnormalities'; or iii) 'abnormal'. Cytologic diagnoses from abnormal CSF were classified into categories as: a) a specific disease; b) multiple possible diagnoses requiring different treatments; c) multiple possible diagnoses all requiring a single type of therapeutic intervention. CNS histology diagnoses were then grouped into the same categories using standardized criteria.

8 CSF samples were not of diagnostic quality. 13 CSF samples had no cytologic abnormality; corresponding histology indicated: no CNS lesion (n = 2); a CNS lesion with insufficient information for treatment (n = 4); infectious disease (n = 2); neoplasia (n = 2) and compression (n = 3). For the 36 samples with cytologic abnormalities, 1 CSF cytology indicated a definitive diagnosis of storage disease. Histologic diagnosis included storage disease as one of multiple differentials. 1 CSF cytology suggested only infectious differential diagnoses but GME was diagnosed on histology. 34 CSF samples were compatible with multiple differential diagnoses requiring different treatments. Of these, histologic diagnosis was neoplasia (n = 8); immune mediated disease (n = 6); infectious (n = 8) and myelomalacia (n = 1) or did not provide sufficient information to treat (n = 11). A comparison group of patients that survived for more than 14 days after CSF analysis showed no difference in the number of CSF samples providing either a definitive diagnosis or a group of diagnoses all requiring the same treatment.

Overall, diagnostic quality CSF samples had a sensitivity of 77% for detection of a lesion in CNS but only 2 (4%) CSF samples provided sufficient information to suggest a treatment modality, and this would have lead to incorrect choice of therapy in 1 of the 2 cases.

This study suggests that although CSF cytology may aid in confirming an inflammatory / infectious CNS process, it rarely provides sufficient information to suggest therapy. Given the increased interest in stereotactic brain biopsy, it is also interesting to note that 30% of CNS post mortem histology results did not give sufficient information for selection of therapy.

## ABSTRACT N-3

**THE PHARMACOKINETICS OF CYTARABINE IN DOGS WHEN ADMINISTERED VIA SUBCUTANEOUS AND CONTINUOUS INTRAVENOUS INFUSION ROUTES.** KI Crook, PJ Early, KR Munana, R Gallagher, K Messenger, MG Papich, JA Nettifee-Osborne. North Carolina State University College of Veterinary Medicine, Raleigh, NC

The objective of this study was to compare the pharmacokinetics of cytarabine (CA) in 6 healthy dogs following intravenous constant rate infusion (CRI) and subcutaneous (SC) administrations. The study was a two-period randomized cross-over design in which each dog received a SC cytarabine injection of 50 mg/m$^2$ and a CRI of 25 mg/m$^2$/hr over 8 hours, with a washout period of 7 days between treatments. Blood samples were collected for 16 hours after initiation of the CRI and for 8 hours after SC injection. Plasma cytarabine concentrations were measured by HPLC. Pharmacokinetic parameters were determined with a compartmental model with the input as either a zero-order CRI, or first-order absorption from the SC dose. The terminal half life (T ½) of CA was 1.35 ±0.3h and 1.15 ± 0.13h after SC administration and CRI, respectively. The mean peak concentration (Cmax) after SC administration was 2.88μg/ml. For CRI administration the Cmax was 2.89μg/ml. Volume of distribution was 0.66 ± 0.07 L/kg. Mean concentrations were sustained above 1.5 μg/mL throughout the entire 8 hour CRI administration, whereas they were sustained above this level for only 2 hours after SC administration. Therefore, steady state achieved with the cytarabine CRI produces a more consistent and prolonged exposure of this agent in the plasma, which is likely to produce an equilibrium between blood and the central nervous system, compared to the poorly sustained concentrations after SC administration. Therefore, IV administration via CRI may have an advantage for treating CNS disease in dogs.

## ABSTRACT N-4

**CEREBROSPINAL FLUID CLUSTERIN IS A POTENTIAL BIOMARKER OF CANINE NEURODEGENERATIVE DISORDERS.** INF Shafie[1], M McLaughlin[1], P Montague[2], PEJ Johnston[1], J Penderis[1], TJ Anderson[1]. [1]School of Veterinary Medicine, [2]Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

The diagnosis of canine neurodegenerative disorders relies on a combination of clinical evaluation, imaging modalities and, if available, genetic assessment and often requires neuropathological examination for confirmation. Despite the continual improvement in the range of diagnostic tests, confirmation of specific diagnosis for canine neurodegenerative disorders such as degenerative myelopathy (DM) can be elusive in the clinic, complicating decision-making and therapeutic management. The purpose of this study was to explore the potential of canine cerebrospinal fluid (CSF) as a source of biomarkers for supporting clinical diagnosis of DM.

Clinical material for this study was derived from cases undergoing routine neurological investigation at the University of Glasgow Small Animal Hospital, with ethical approval. All cases were diagnosed using routine techniques including haematology, biochemistry, CSF analysis and advanced imaging. Additionally, all cases were genotyped as to the presence of the *SOD1* mutation that is associated with DM; using an in house protocol. CSF protein profiling by gel electrophoresis demonstrated an abundant protein in DM that was subsequently identified by mass spectrometry as clusterin (also known as apolipoprotein J). Initial semi-quantitative Western blot analysis of canine CSF confirmed that clusterin was significantly elevated in DM (n = 5) compared to IE (n = 7) ($p < 0.01$). A further comparison across a range of neurological conditions found that clusterin was elevated in DM (n = 4) and chronic intervertebral disk disease (cIVDD) (n = 4) compared to IE (n = 7) (DM vs. IE, $p < 0.001$; cIVDD vs. IE, $p < 0.05$) and meningitis (n = 8) (DM vs. meningitis, $p < 0.01$; cIVDD vs. meningitis, $p > 0.05$). There was no significant difference in CSF clusterin between DM and cIVDD. An investigation of plasma clusterin level in DM (n = 5) and IE (n = 7) found no significant difference in the protein level, although a proportion of DM cases (3/5) demonstrated a gel shift which may indicate a post translational modification.

Clusterin mRNA level in the thoracic spinal cord of DM and control cases (dogs unaffected by neurological diseases) was compared by RT-PCR. For this comparison tissue was derived from archival post mortem material and *SOD1* genotyping was undertaken to support the histopathological diagnoses. CSF samples were not available for these cases. The mean mRNA level was elevated by 42% in DM (n = 4) compared to control cases (n = 4), a difference bordering on statistical significance ($p = 0.05$).

These observations suggest CSF clusterin may be considered as a potential biomarker for canine neurodegenerative conditions, potentially those associated with neuronal dysfunction and death. Clusterin may therefore represent one component in a panel of emerging biomarkers that may combine to distinguish specific neurodegenerative disorders such as DM in the clinic.

## ABSTRACT N-5

**L-2-HYDROXYGLUTARIC ACIDURIA IN YORKSHIRE TERRIER DOGS: CHARACTERIZATION OF THE MOLECULAR DEFECT.** D Sanchez[1,2], J Mascort[2], C Jakobs[3], GS Salomons[3], R Artuch[4], A Lujan Feliu-Pascual[2]. [1]Animal Health Trust, Newmarket, UK, [2]Hospital ARS Veterinaria, Barcelona, Spain, [3]VU University Medical Center, De Boelelaan, Amsterdam, The Netherlands. [4]Hospital San Joan de Déu, Barcelona, Spain

L-2-hydroxyglutaric aciduria (L-2-HGA) represents a recently defined inborn error of metabolism with a variety of clinical neurological deficits including seizures, head tremors and ataxia.

Two female Yorkshire terrier dogs were evaluated because of generalized tonic-clonic seizures. The dogs underwent a magnetic resonance imaging study of the brain. T1-weighted, T2-weighted and fluid-attenuation inversion recovery sequences were obtained in different planes. These images revealed bilaterally symmetrical, diffuse regions of grey matter hyperintensity on T2-weighted and FLAIR images, which were most prominent in the parietal cerebral cortex, thalamus, mesencephalon and cerebellum. Postcontrast T1-weighted sequences showed no contrast enhancement. Based on the imaging findings, differential diagnoses included metabolic or toxic encephalopathies.

To investigate the possibility of an intrinsic metabolic disorder, urinary organic acid and amino acid profiles were quantified by gas chromatography-mass spectroscopy and were consistent with diagnosis of L-2-HGA.

In both affected dogs a homozygous pathogenic mutation at the translation initiation codon of the *L2HGDH* gene was detected (c.1A > G; p.Met1?) confirming the diagnosis L-2-HGA at the DNA level. To our knowledge these are the first two cases described of L-2-HGA in Yorkshire terriers, with a different mutation to the Staffordshire bull terriers.

## ABSTRACT N-6
## DIAGNOSTIC VALUE AND DISCRIMINATORY ABILITY OF PROTON MAGNETIC RESONANCE SPECTROSCOPY FOR INTRACRANIAL NEOPLASIA IN DOGS.

KR Mikoloski, PA March, D Faissler. Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA

Metabolic profiles have been developed using proton magnetic resonance spectroscopy ($^1$H-MRS) in humans to aid in distinguishing types of intracranial neoplasia. The goal of our study was to determine whether $^1$H-MRS was useful in discriminating different types of intracranial neoplasia in dogs.

Ten dogs with intracranial neoplasia and 2 normal dogs underwent single voxel $^1$H-MRS in addition to a standard MRI. Spectra were obtained at echo times of 135 ms (long TE) and 30 ms (short TE). A manual, interactive shimming protocol was used. Metabolites were quantified as areas beneath their respective peaks. Creatine (Cr), Choline (Cho), and N-acetyl aspartate (NAA) were measured on long TE spectra. Cr, myoinositol/glycine (mIG), and Cho were measured on short TE spectra. At short TE, the NAA peak was partially obscured in many cases by a broad overlapping glutamine/glutamate/macromolecule complex (Glx/MM), making precise measurement difficult. Lactate (Lac), lipids, and alanine (Ala) were also subjectively evaluated.

A histopathologic diagnosis with either necropsy or surgical biopsy was obtained in seven dogs (3 meningiomas, 2 gliomas, 2 metastatic tumors). Presumptive diagnoses were made in the remaining three dogs based on MRI and clinical findings (probable meningioma in 1 dog and probable glioma in 2 dogs).

mIG resonance on short TE spectra was over 4 times greater in gliomas than meningiomas (1.98 versus 0.45) as compared to 0.86 in normal dogs. The Glx/MM/NAA complex on short TE spectra was subjectively more prominent in meningiomas than in gliomas. Ala was noted in one dog with a meningioma on short TE spectra. On long TE spectra, both Cr and NAA were reduced in all tumor groups compared to normal dogs. The mean Cr resonance at long TE was lower in meningiomas compared to gliomas (0.98 versus 1.21). Cho concentrations on long TE spectra were greater in dogs with intracranial neoplasia compared to normal dogs (3.84 versus 2.83). Lac and lipid peaks were subjectively higher in dogs with metastatic neoplasia than in dogs with primary neoplasia.

Based on the results of this study, meningiomas and gliomas appear to have some important differences on $^1$H-MRS that may help differentiate these tumors. The more prominent and consistent differences were seen in mIG and Glx/MM metabolites on short TE spectra. mIG was greater in dogs with gliomas than in normal dogs and in dogs with meningiomas. The Glx/MM metabolites were greater in dogs with meningiomas than in dogs with gliomas. $^1$H-MRS spectra may be a valuable method to non-invasively differentiate meningiomas from gliomas. More cases will be needed to determine if these differences in their metabolite profiles are statistically significant.

## ABSTRACT N-7
## MAGNETIC RESONANCE IMAGING FEATURES OF INTRACRANIAL GRANULAR CELL TUMORS IN SEVEN DOGS.

CC Anwer[1], KM Vernau[1], RJ Higgins[1], PJ Dickinson[1], BK Sturges[1], RA LeCouteur[1], RT Bentley[2], ER Wisner[1]. [1]University of California – Davis, Davis, CA, [2]Purdue University, West Lafayette, IN

Intracranial granular cell tumors (GCTs) are rarely reported in dogs. Histopathological descriptions exist, however magnetic resonance (MR) imaging features have been described in only two dogs. The goal of this retrospective study is to describe the MR characteristics of intracranial GCTs in a larger number of dogs.

Seven dogs with histologically confirmed intracranial GCTs and complete MR imaging were studied. MR images were evaluated for tumor location, size, mass effect, T1- and T2-weighted signal intensity, contrast enhancement, and peritumoral edema. In MR images of 6/7 patients, GCTs were well-defined and extra-axial, with a plaque-form, sessile distribution involving the meninges. These six tumors were located along the convexity of the cerebrum, the falx cerebri, or the ventral floor of the cranial vault and were mildly hyperintense on T1-weighted images, and iso- to hyperintense on T2-weighted images. A moderate to marked degree of peritumoral edema and mass effect was evident in all six patients. Interestingly, 1/7 dogs had a small suprasellar GCT that was isointense on T1- and T2-weighted images, and did not have a plaque-form shape, peritumoral edema, or mass effect.

In summary, hyperintensity on T1-weighted MR images, plaque-form distribution, and marked peritumoral edema and mass effect were consistent cerebral GCT imaging features in 6/7 dogs. However these findings are not unique to GCTs. In addition, MR imaging features of the solitary suprasellar GCT differed from the other GCTs in the study.

## ABSTRACT N-8
## SIGNALMENT, CLINICAL FINDINGS, MAGNETIC RESONANCE IMAGING FEATURES AND SURGICAL OUTCOMES WITH HISTOPATHOLOGICALLY CONFIRMED EPIDURAL AND SUBDURAL SPINAL CORD HEMATOMA IN DOGS.

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Epidural spinal cord hematoma has been described in veterinary medicine in association with neoplasia, intervertebral disc disease, and snake envenomation. In human medicine there are rare reports of spontaneous epidural or subdural spinal cord hematoma where there was no known cause. Spontaneous spinal cord hematoma has not been described in veterinary medicine. The purpose of this study is to describe the signalment, clinical findings, magnetic resonance imaging features and surgical outcomes in histopathologically confirmed epidural and subdural spinal cord hematomas in dogs with no identified underlying etiology.

Five dogs were included with a histopathologic diagnosis of epidural or subdural spinal cord hematoma not associated with an underlying cause. Magnetic resonance imaging and surgical outcomes were evaluated. Large breed dogs (> 25 kg) accounted for 4/5 of the cases. There were 3 spayed females and 2 neutered males. The mean age was 5.4 years. On presentation, most dogs (4/5) were ambulatory with paresis and ataxia and only one patient was non-ambulatory and paretic. In most dogs (4/5), focal spinal pain was elicited on examination. All patients had an acute onset and then progression of clinical signs. There was a range of 1-13 days between the onset of clinical signs to magnetic

resonance imaging. Hematoma was located at the vertebral column level C2, C4-5, C5, T13-L1 and L5.

Magnetic resonance imaging and surgery showed 3 patients had epidural and 2 patients had subdural hematoma formation. All five dogs had normal signal within the spinal cord and variable spinal cord compression ranging from 20-50%. All hematomas had mild to moderate T2W hyperintensity and mild to strong contrast enhancement. They appeared isointense in 3 cases and mildly hyperintense in 2 cases on the T1W pre-contrast sequences. Prior to surgery and histopathology the primary differential in most cases was neoplasia.

The underlying etiology of the hematomas was not identified in all cases. All dogs had adequate platelet numbers. A coagulation panel performed on one dog was normal. Two dogs had intraoperative cultures which were negative.

All dogs underwent surgical decompression with dorsal or hemilaminectomy and all patients returned to normal or near normal neurologic function following surgery. Follow-up on the patients (ranging between 14-188 days) shows no progression of neurologic symptoms or conditions associated with increased bleeding. The prognosis with spontaneous spinal cord epidural and subdural hematoma with surgical decompression appears to be favorable.

**ABSTRACT N-9**

**MRI ASSESSMENT OF DOGS WITH DESCENDING TRANSTENTORIAL AND/OR FORAMEN MAGNUM BRAIN HERNIATIONS.** JR Barker, D Faissler, O Taeymans. Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA

The aim of this study was to develop a scoring system of MRI images assessing backwards movement of the brain in the caudal fossa caused by supratentorial space occupying lesions.

A semi-quantitative approach determining CSF filling in eight regions, and a quantitative measurement of four different parameters were designed. MRI images were examined for lesion location and lateralization. Total brain and lesion volume, surrounding edema, and relative volume of the mass were calculated. Clinical signs were grouped into seizure, abnormal cranial nerve function, cranial nerve dysfunction combined with abnormal gait and endocrine disease. Pairwise data analysis was performed comparing affected with unaffected dogs using the SPSS 19 software package.

81 affected and 81 control dogs were analyzed. Sex and breed distribution were similar in both groups. All semi-quantitative and the quantitative parameters of tentorial angle, foramen magnum line and brainstem ratio showed a significant difference between study and control dogs. Tentorial angle, cerebellar height, length, their ratio, spinal cord height and brainstem to spinal cord ratio were significantly correlated to the degree of CSF filling. Lesions in the frontal or olfactory lobes caused significantly less herniation when compared to masses in other brain areas. Total and relative lesion volumes were significantly correlated to all semi-quantitative measurements, tentorial angle and cerebellar length.

In conclusion, our semi-quantitative measurements and tentorial angle best determine the magnitude of brain herniation. The ratio of lesion and edema volume to brain volume can predict degree of herniation best when used in conjunction with lesion location.

**ABSTRACT N-10**

**KINEMATIC GAIT ANALYSIS USING 3-D MOTION CAPTURE IN DOBERMAN PINSCHERS WITH AND WITHOUT CERVICAL SPONDYLOMYELOPATHY.** K Foss, RC da Costa. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

The most widely used method for determining the response to treatment in patients with cervical spondylomyelopathy (CSM) is based off of the owner and clinician's perception of the gait. This form of evaluation is highly subjective and can suffer from observer bias. The purpose of this study was to utilize digital video motion capture to compare multiple kinematic parameters between Doberman Pinscher dogs with and without CSM.

Nineteen Doberman Pinschers were prospectively studied; 10 clinically normal dogs and 9 with CSM. Neurologic examinations were performed in all dogs prior to enrollment. Clinically normal dogs also underwent transcranial magnetic stimulation to further exclude any underlying neurologic abnormalities. All CSM-affected dogs had their diagnosis confirmed with a 3.0 T MRI. All dogs were fitted with a lycra bodysuit and 32 reflective markers representing specific anatomic landmarks from all four limbs, head and trunk were applied. 3-D motion capture was performed with 15 infrared cameras in a designated capture space using the Vicon8i motion capture system.

Using Vicon iQ 2.0 software (Vicon, CA), the markers were used to reconstruct a 3-D stick diagram representation of each dog. This representation was then used to measure multiple parameters of interest. Parameters evaluated included stride duration, stride length, stride height, maximal and minimal spinal angles, elbow and stifle flexion and extension, and maximum and minimum distances between the thoracic and pelvic limbs. At least 45 seconds of high quality data capture were used for statistical analyses. The means of all parameters were compared between normal and CSM-affected Dobermans using a random effects linear regression model.

Due to space constraints only portion of results will be presented and discussed. Minimum thoracic limb distance was significantly smaller ($P = 0.024$) in CSM-affected dogs (mean = 116 mm) versus normal dogs (mean = 146 mm), as was maximum thoracic limb distance ($P = 0.001$) with a mean of 184 mm in CSM-affected dogs and 222 mm in normal dogs. Additionally, stride duration in the thoracic limbs was also significantly smaller (left thoracic: $P = 0.013$; right thoracic $P = 0.016$) in CSM-affected dogs (mean = 0.7 sec) when compared to normal dogs (mean = 0.8 sec).

In conclusion, the thoracic limb distances and thoracic limb stride duration were found to be significantly smaller in CSM-affected Doberman Pinschers when compared to normal Dobermans. Interestingly, these findings suggest that when using computerized kinematic gait analysis to compare the gait between normal and CSM-affected Dobermans, the thoracic limbs appear to reveal more consistent differences than the pelvic limbs.

**ABSTRACT N-11**

**THE VACUUM PHENOMENON IN INTERVERTEBRAL DISC DISEASE OF DOGS BASED ON COMPUTED TOMOGRAPHY IMAGES.** MK Müller[1], E Ludewig[1], G Oechtering[1], M Scholz[2], T Flegel[1]. [1]University of Leipzig, Department of Small Animal Medicine, Leipzig, Germany;, [2]University of Leipzig, Institute for Medical Informatics, Statistics and Epidemiology, Leipzig, Germany

The term "vacuum phenomenon" is used to describe accumulation of gas within tissues such as degenerated intervertebral discs. Thus this phenomenon is sometimes used, to localise a herniated discs. The purpose of this study therefore was 1. to determine the frequency of the vacuum phenomenon in dogs surgically treated for intervertebral disc diseases, 2. to evaluate factors influencing the frequency of the vacuum phenomenon and 3. to determine the value of the vacuum phenomenon for localizing disc herniation.

Computed tomography scans of dogs with confirmed intervertebral disc herniation were examined for a vacuum phenomenon. Patients were included if a computed tomography with or without subarachnoidal contrast was performed pre-surgically and the site of radiologically diagnosed disc herniation could be confirmed by the surgical report, immediate postsurgical computed tomography images or necropsy report. Inclusion criteria were met by 187 dogs. Dogs were divided for statistical analysis into the following subgroups: chondrodystrophic dogs (61%), non chondrodystrophic dogs (15%) and such of unclear classification (24%).

The median number of scanned discs was 14 (range 4-26). Vacuum phenomenon was found in 19.8 % of dogs. The prevalence in subgroups was 14.9% in chondrodystrophic dogs, 34.5%

in non chondrodystrophic dogs, and 24.4% in dogs of unknown classification respectively. The frequency of vacuum phenomena was significantly higher in chondrodystrophic than in non chondrodystrophic dogs (P= 0.03). Age has a significant influence on the appearance of the vacuum phenomenon. With every additional year of age the relative risk to show a vacuum phenomenon increases by 15 % (P = 0.04).

The vacuum phenomenon appeared in the currently herniated intervertebral disc in 64.1 %. The frequencies in the subgroups of chondrodystrophic, non chondrodystrophic dogs and such of unknown classification were 67%, 40%, and 82% respectively. The correspondence between vacuum phenomenon and herniated disc in dachshunds only was 86%. Non chondrodystrophic dogs were less likely to show a vacuum phenomenon in the currently herniated disc (P = 0.03). Age does not have a significant influence on the concurrence between the site of the phenomenon and the actual herniated disc except in chondrodystrophic dogs, where the relative probability increases by 22% every year (P = 0.04).

In conclusion, vacuum phenomenon is a frequent but inconsistent finding in intervertebral disc disease in dogs. It does, however, not allow localizing the currently herniated disc.

## ABSTRACT N-12

**MUTATION ASSOCIATED WITH NEONATAL CEREBELLAR CORTICAL DEGENERATION IN BEAGLE DOGS IDENTIFIED BY GENOME-WIDE MRNA SEQUENCING.** E Beltran, O Forman, C Mellersh, J Stewart, L De Risio. Animal Health Trust, Newmarket, UK

Neonatal cerebellar cortical degeneration is a neurodegenerative disease described in several canine breeds including Beagle dogs, for which autosomal recessive mode of inheritance has been suggested. The affected dogs are unable to ambulate normally since the onset of walking and the main pathological finding includes Purkinje neuron loss with swollen dendritic processes. The aim of the study was to investigate whether a mutation in one of the candidate genes was responsible for this disease.

A four-week old Beagle dog presented to the Animal Health Trust with progressive signs of cerebellar ataxia. The neuroanatomic localization was to the cerebellum. Hematology and comprehensive biochemistry were normal. The owner elected euthanasia. Histopathology was consistent with neonatal cerebellar cortical degeneration. Genome-wide mRNA sequencing (mRNA-seq) from post-mortem cerebellum tissue was used as a method of candidate gene sequencing. After analysis of the canine orthologues of spinocerebellar ataxia associated genes from human studies, we identified a homozygous 8 bp deletion in a candidate gene previously associated with spinocerebellar ataxia in humans. Genotype analysis of the sire, dam and six clinically unaffected siblings showed the mutation to fully segregate with the disorder. Previous studies have demonstrated that this protein is critical for Purkinje cell development, and its absence can lead to cell damage through excitotoxicity, explaining the observed Purkinje neuron loss, degeneration of dendritic processes and associated neurological dysfunction. The mentioned mutation has been found, at a low frequency, in the United Kingdom population of Beagles and a DNA test is being developed.

## ABSTRACT N-13

**SURGICAL REMOVAL OF FELINE INTRACRANIAL MENINGIOMAS: CLINICAL FEATURES AND OUTCOME IN 121 CASES (1994-2011).** S Cameron, CW Dewey, M Rishniw. Cornell University College of Veterinary Medicine, Ithaca, NY

Meningiomas are the most common brain tumor reported in cats. The purpose of this study was to report clinical features and outcome of a large group of cats with surgically removed intracranial meningiomas.

Data were collected from 121 cats with intracranial meningioma removed surgically and confirmed histopathologically. Breed, sex, age, clinical signs, duration of clinical signs, drugs used before and after surgery, biopsy results, imaging reports, and outcome were recorded for each case. 83 of the 121 cats were domestic short-hairs with 76 neutered males and 43 spayed females. Body weight ranged from 1.5 to 8.7kg (median 4.95kg). Age ranged from 3.3 to 19.2 years (median age 12.2 years). 81 cats displayed behavior changes, 46 were ataxic, 32 had seizures, 31 had visual deficits, 24 were circling, and 14 were paretic. Duration of neurologic signs ranged from 0 to 23 months (median 1.25 months). The duration of non-neurologic signs ranged from 0 to 18 months (median 2 months). At the time of data collection, 41 cats were alive, 26 were dead or euthanized, and 54 were lost to follow-up. Of the 26 cats that died, 5 were due to causes directly related to the meningioma and/or surgery. 8 cats died due to causes that were thought to be related to the original tumor, surgery, or regrowth of the tumor. The mean survival time for cases, excluding those lost to follow-up 17.8 months (range 0-71 months). Overall, cats with meningiomas treated with surgery alone had a good prognosis.

## ABSTRACT N-14

**EFFECT OF CHLORIDE IN INFUSION FLUID ON SERUM BROMIDE CONCENTRATION IN DOGS.** K Orito[1], K Fukunaga[1], H Matsumoto[1], M Wate[2], M Muto[2], M Saito[2]. [1]Department of Physiology II, [2]Department of Veterinary Surgery II, School of Veterinary Medicine, Azabu University, Sagamihara, Kanagawa, Japan

Bromide is frequently used for the management of epilepsy in canine medicine. Dogs with high dietary chloride content reportedly show a reduction of serum bromide concentration. However, it is unclear how chloride content in infusion fluid affects serum bromide concentration in dogs. It is also unclear whether the chloride lowers serum bromide concentration dependently on the initial serum bromide concentration. In the present study, therefore, the effects of saline transfusion on various serum concentration of bromide were examined in beagles.

Potassium bromide was administered to beagles with a loading dose of 500 mg/kg, PO, and the serum bromide concentration was maintained with a maintenance dose of 25 mg/kg, PO, BID. After stabilization of the serum bromide concentration, saline was infused intravenously at different rates for 5 h. Serum and urine were collected before and 1, 2, 3, 4, 5, 6, and 7 h after the initiation of transfusion. Bromide concentrations of serum and urine were measured using high-performance liquid chromatography and an ion-selective electrode, respectively.

Serum bromide concentration decreased and urine bromide concentration increased when saline was transfused for 5 h. These effects were marked when saline was infused at a higher rate. The rate of reduction of serum bromide concentration was higher when the initial serum bromide concentration was higher.

In conclusion, serum bromide concentration is affected by chloride content of infusion fluid, and the concentration of serum bromide is the determinant of the rate of reduction of serum bromide.

## ABSTRACT N-15

**DOES BODY CONDITION SCORE INCREASE RECOVERY TIME IN DOGS TREATED WITH HEMILAMINECTOMY FOR ACUTE ONSET DISC RUPTURE?** CC Williams, G Barone. Veterinary Medical Center of Long Island, West Islip, NY

Intervertebral disc disease (IVDD) was initially reported in the 1800's, with surgical intervention first being described in the 1950's. One previous study conducted showed a correlation between body dimensions and the risk of IVDD. However, there have been no studies indicating a relationship with body condition and post-operative recovery rate. In this study, we hypothesized that dogs with a higher body condition score (BCS) that underwent hemilaminectomy to repair acute onset intervertebral disc rupture would have a lengthier recovery time.

Medical records from fifty-one dogs that had undergone hemilaminectomy to repair acute IVDD (surgically confirmed) in the

thoracolumbar spine were reviewed. Parameters evaluated included signalment, length of clinical signs prior to presentation, length of recovery time, and neurologic grades pre and post-op. A BCS grading scheme of 1 through 9 was used for all fifty-one dogs. Finally, the BCS and time to recovery was compared statistically in all dogs. Recovery was defined as "ambulation without assistance".

Results of the study revealed that there was a significant association between BCS and time to recovery. The dogs were 7.62 times more likely to have recovered at the initial 3 to 4 week follow-up if they had a BCS of six or less.

In conclusion, this study showed that as weight increased, the time to recovery post hemilaminectomy surgery, also increased.

**ABSTRACT N-16**
**IMMUNOHISTOCHEMICAL QUANTIFICATION OF INTERLEUKIN-6 AND INTERLEUKIN-8 EXPRESSION IN CANINE INTRACRANIAL MENINGIOMAS.** S Al-Nadaf[1], SR Platt[2], M Kent[2], N Northrup[2], EW Howerth[2]. [1]North Carolina State University, College of Veterinary Medicine, Raleigh, NC, [2]University of Georgia, College of Veterinary Medicine, Athens, GA

Meningiomas are the most common intracranial tumors of dogs. Although surgical excision is the primary treatment available, recurrence remains prevalent. The expression of pro-inflammatory cytokines, interleukin-6 (IL-6) and interleukin-8 (IL-8), has been detected in human intracranial meningiomas, and these cytokines have been shown to both promote and inhibit growth of the tumor; additionally, the over-expression of IL-6 has been recorded as a marker of malignancy in human gliomas. However, meningiomas in dogs have yet to be investigated for IL-6 and IL-8 expression despite sharing similar histological appearance to human meningiomas.

The purpose of this study was to evaluate IL-6 and IL-8 immunohistochemical expression in canine intracranial meningiomas.

Immunohistochemistry was performed on 19 formalin-fixed, paraffin-embedded canine intracranial meningioma samples using mouse anti-canine IL-6 monoclonal antibody and mouse anti-canine IL-8 monoclonal antibody. Normal canine tonsil lymphoid tissue, canine carcinoma tumor tissue and a canine malignant histiocytic cell line (DH82 cells) challenged with lipopolysaccharide served as controls. Each tumor was evaluated for staining intensity and the percent area of positive staining cells using a subjective scoring system. The percent area of positive staining was scored as follows: 0, 0% of cells stained; 1, 1-30% of cells stained; 2, 31-60% of cells stained; 3, 61-100% of cells stained. Intensity was scored as: 0, negative expression; 1, weak expression; 2, moderate expression; 3, marked expression.

Of the 19 meningiomas, immunochemistry staining for IL-6 expression was positive in five (26%), and IL-8 expression was positive in none (0%). In the five samples positive for IL-6 expression, the percent staining area was greater than 61% (score of 3) with two (40%) having weak (score of 1) staining intensity and three (60%) having moderate (score of 2) staining intensity.

In conclusion, IL-6 expression, but not IL-8, was demonstrated in a subset of canine intracranial meningiomas. Further studies are needed to determine the role of IL-6 in canine intracranial meningioma tumorigenesis and whether IL-6 expression in a subset of these tumors could be used as a potential cell marker and as targeted immunotherapy.

**ABSTRACT N-17**
**A NOVEL ANTI-CANINE CD133 ANTIBODY COLOCALIZES WITH GFAP IN NORMAL AND NEOPLASTIC CANINE BRAIN TISSUE.** D York, RJ Higgins, RA LeCouteur, PJ Dickinson. University of California Davis, Davis, CA

CD133 is a penta-transmembrane protein that has been proposed as a potential marker for a variety of stem cells and also brain tumor stem-like cells. However there is much controversy over its specificity and value in relation to therapeutic targeting. Expression based on a variety of CD133 antibodies has been conflicting and is complicated by alternative transcription and variable glycosylation patterns.

We developed a novel polyclonal antibody targeted against a canine specific 16aa peptide in the intracellular C-terminal domain of the canine CD133 protein. On western blotting, the antibody recognizes an approximately 80kDa protein in canine adult brain and kidney, neonatal brain, canine glioma cell lines. Minimal signal was seen in murine tissues. We compared the antibody to one previously reported in canine tissue (Clone 13A4) and found strong staining only in murine tissues with 13A4.

Immunohystochemistry revealed staining of astrocytic-like cells in normal canine and murine brain and orthotopic canine glial tumors. Neurospheres derived from neonatal dog brain also had positive staining. Almost all cells in the normal canine brain staining positive for CD133 co-stained for GFAP, as did neurospheres and cells within orthotopic tumors. Costaining was also seen in peritumoral tissue from human glioblastoma multiforme. GFAP has been reported as a marker of adult neural stem cells, however CD133/GFAP co-localization differs from previous studies where GFAP did not generally co-stain with CD133. These findings have potential implications for the use of CD133 as a marker for stemness and therapeutic targeting in canine brain tumors.

**ABSTRACT N-18**
**CLINICOPATHOLOGIC AND MAGNETIC RESONANCE IMAGING CHARACTERISTICS OF HYPERTENSIVE ENCEPHALOPATHY IN TWO CATS AND TWO DOGS.** J O'Neill[1], M Kent[1], E Glass[2], SR Platt[1]. [1]College of Veterinary Medicine, University of Georgia, Athens, GA, 30602, [2]Red Bank Veterinary Hospital, Tinton Falls, NJ 07724

Two dogs and two cats were evaluated for an acute onset of neurologic dysfunction. Signs were referable to the prosencephalon consisting of abnormal mentation, recumbency, and blindness. All cases had consistent systemic hypertension ranging from 180mm/Hg to 260mm/Hg as measured using Doppler ultrasonography. Magnetic resonance imaging (MRI) of the brain disclosed non-contrast enhancing, ill-defined T2-weighted hyperintensities in the white matter of the cerebrum in the areas of the frontal, parietal, temporal, and occipital lobes. Lesions were observed in the caudate nuclei and thalamus in two animals, respectively. There was scant contrast enhancement of the caudate nuclei in one case. Retinal lesions consisting of retinal hemorrhages and or detachment were observed in three animals. Hypertension was presumed secondary to renal disease in three animals; a primary underlying disorder was not identified in one animal. Normalization of blood pressure was achieved within the first 3-5 days with amlodipine (0.1mg/kg Q12hr) alone or in combination with enalapril (0.5mg/kg PO Q24hr). In one cat, hypertension spontaneously resolved. In three cases, neurological improvement occurred within 24-48 hours of normalization of blood pressure. The presumptive diagnosis of hypertensive encephalopathy is supported by the MRI findings similar to humans and neurological dysfunction coincident with systemic hypertension in which the neurological dysfunction improves with treatment of hypertension. All cases were alive and doing well at follow-up. Follow-up times ranged from 8.5months to 18months. The prognosis appears good for the resolution of neurological deficits with normalization of blood pressure in animals with hypertensive encephalopathy.

**ABSTRACT N-19**
**RELATIONSHIP BETWEEN SPINAL CORD SIGNAL CHANGES AND CLINICAL AND MRI FINDINGS IN DOGS WITH CERVICAL SPONDYLOMYELOPATHY – 102 CASES.** RC da Costa. Dpt. of Vet Clinical Sciences, College Vet. Medicine, The Ohio State University, Columbus, OH

Spinal cord signal changes (SCSC) are frequently seen in the MR (magnetic resonance) images of dogs with cervical spondylomyelopathy (CSM). However, little is known about the factors

causing these changes and their relationship to clinical and MR findings. This study aimed to evaluate these relationships in large and giant breed dogs with CSM.

Medical and radiological records of dogs having CSM were searched between 2003 and 2011. Inclusion criteria included a definitive diagnosis of CSM achieved by MR (using high fields units, either 1.5 or 3.0 T) in a large or giant breed dog. The following information was collected from the medical records: breed, age, history (acute $\leq$ 5 days, chronic $>$ 6 days), and severity of neurological findings (1 = neck pain, 2 = mild ataxia, 3 = moderate ataxia, 4 = severe ataxia, 5 = non-ambulatory). All MRIs were reviewed and information regarding the presence or absence of SCSC, as well as the location, direction and severity (mild $\leq$ 25% cord diameter, moderate 25-50%, severe $>$ 50%) of compressive lesion were recorded. Statistical analyses were performed with Fisher's exact text for categorical variables and Wilcoxon rank-sum test for continuous variables.

One hundred and two dogs met the inclusion criteria. Sixty two were large breed dogs and 40 were giant breed dogs. Forty of the 62 large dogs were Doberman pinschers and 21 of the giant breed dogs were Great Danes. The remainder was composed of several breeds. Overall 57 dogs (55.9%) showed SCSC; 36 (58.1%) of the large breed dogs and 21 (52.5%) of the giant breed dogs. The age of dogs with and without SCSC was 3.2 ± 0.9 and 2.6 ± 1.0, respectively ($p = 0.41$). The mean score of neurological deficits in dogs with SCSC was 3.2 ± 0.9, whereas in dogs without SCSC it was 2.6 ± 1.0 ($p = 0.001$). Dogs with SCSC more commonly had a chronic history (80.7% vs. 62.2% for those without SCSC) ($p = 0.046$). The location of the compressive lesion was not significant ($p = 0.45$) as the compression was found at either C5-6 or C6-7 in 89.4% of dogs with, and 81.8% of dogs without SCSC. The severity of the compression proved to be significant ($p = 0.001$) as 89.5% dogs with SCSC had severe (47.4%) or moderate (42.1%) compressions, whereas only 48.9% of dogs without SCSC had severe (13.3%) or moderate (35.6%) compressions. The direction of compression (ventral, dorsal/dorsolateral, or lateral/bilateral) was not significant.

The results indicate that SCSC are seen in both large and breed dogs with CSM, with an overall incidence of 55.9%. SCSC were significantly more common in dogs with a chronic history, more severe neurological deficits, and with moderate or severe spinal cord compressions. The location and direction of the compressive lesions had no influence on the development of SCSC in dogs with CSM.

**ABSTRACT N-20**
**FORCE PLATE GAIT ANALYSIS IN DOBERMAN PINSCHERS WITH AND WITHOUT CERVICAL SPONDYLOMYELOPATHY.** K Foss[1], RC da Costa[1], M Allen[1], PJ Rajala-Shultz[2]. [1]Department of Veterinary Clinical Sciences, [2]Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA

Currently, the most accepted means of evaluating the response to treatment in a patient with cervical spondylomyelopathy (CSM) has been a subjective measure based on the owner and clinicians' perception of the patient's gait. The goal of this study was to identify differences in force plate (FP) parameters between Doberman Pinschers with and without CSM and then use this information to subsequently develop a more reliable system for detecting, quantifying, and differentiating gait abnormalities in CSM.

Nineteen Doberman pinschers were prospectively studied; 10 clinically normal dogs and 9 with CSM. Neurologic examinations were performed in all dogs. Clinically normal dogs underwent transcranial magnetic stimulation to further exclude any underlying neurologic abnormalities. CSM-affected dogs had their diagnosis confirmed with a 3.0 T MRI. Force plate analysis was performed in all four limbs of all dogs using a stationary force plate and computer system (Kistler Model 9687A). At least 4 runs of ipsilateral limbs were collected from each dog. Parameters evaluated included the peak vertical force (PVF), peak vertical impulse (PVI), peak braking force (PBF), peak braking impulse (PBI), peak propulsive force (PPF), peak propulsive impulse (PPI), peak mediolateral force (PMLF), and peak mediolateral impulse (PMLI). All force peaks were represented as a percentage of body weight and the mean value of each parameter was calculated. In addition, the coefficient of variation (CV) within each limb was also calculated for each individual parameter. A repeated measures approach was used to analyze the data.

In regard to peak forces and impulses, there were no significant differences between PVF, PVI, PBF, PBI, PPF, PPI, or PMLI in thoracic or pelvic limbs in affected versus normal dogs. PMLF of all limbs was significantly higher in normal dogs compared to CSM-affected dogs ($P = 0.0062$). By performing an analysis related to variability using the CV, there was significantly more variability of PVF in the CSM-affected dogs compared to normal dogs ($P = 0.0045$). In addition, variability of PVF in the thoracic limbs was significantly higher in CSM-affected Dobermans compared to the normal ones ($P = 0.0019$).

In conclusion, the variability of PVF in all four limbs and in particular the thoracic limbs, seems to consistently distinguish clinically normal Dobermans from those with CSM. Even though ataxia and weakness are manifested primarily in the pelvic limbs of dogs with CSM, this data suggests that FP analysis should evaluate all limbs, and the thoracic limbs may be more sensitive to differences than the pelvic limbs.

**ABSTRACT N-21**
**CHARACTERIZATION OF A NOVEL CANINE GBM-DERIVED CELL LINE (G06A) AND ORTHOTOPIC TUMORS IN MICE.** D York, RJ Higgins, RA LeCouteur, PJ Dickinson. University of California Davis, Davis, CA

Glioblastoma multiforme (GBM), grade IV astrocytoma, is the most common primary brain tumor in humans, and carries a poor prognosis with current therapies. Canine spontaneous brain tumors bear striking similarities to their human tumor counterparts and may provide a valuable translational model system for novel therapies. Veterinary preclinical assessment and development of targeted therapies has been hampered by a lack of suitable canine glioma cell lines and murine xenograft models. We have established a novel canine glioma cell line (G06A) derived from a spontaneous canine GBM. The G06A cells grow adherently in standard serum media, with an average doubling time of 22 hours. Cells express wild type p53 and overexpress IL13R2 consistent with the original tumor.

Intracranial implantation of $1e^6$ G06A cells resulted in orthotopic tumors in 7/7 NOD *scid* gamma (NSG) mice and 3/3 NCr Nude mice 8-13 months following implantation. Routine histopathology revealed extensive, invasive tumors consistent with a high grade, mixed glial phenotype. Tumor invasion occurred throughout the neuropil involving both white and grey matter and was not limited to perivascular spaces. Both oligodendroglial and astrocytic characteristics were present, reflective of the original tumor. Based on immunohistochemistry, cells within the tumor mass had positive staining for GFAP, Olig2, Nestin, and CD133. Although the tumor latency period makes *in vivo* therapeutic testing problematical, the cell line may be valuable for *in vitro* screening and investigation of factors involved in tumor progression and host/tumor oncogenic signaling.

# SMALL ANIMAL – NUTRITION / METABOLISM

**ABSTRACT NM-1**
**NUTRITIONAL IMMUNOMODULATION: BOVINE COLOSTRUM FOR IMMUNE & GUT HEALTH IN DOGS.** E Satyaraj, A Reynolds. Nestle Purina Research, St. Louis, MO

While the need for colostrum in neonates is well established, the immunomodulatory effect of feeding bovine colostrums [BC] to adult humans is gaining increased attention. However, no such studies in dogs have been reported. The aim of this study was to evaluate the immunomodulatory effect of BC in dogs.

The trial was conducted in two phases: Pre-test [8 wks] and Test [40 wks]. 24 dogs [mean age 2.5 yrs] were randomized into two groups. In 'Pre-test' phase both groups were fed a nutritionally complete diet. At the end of 'Pre-test' phase all dogs received a Canine Distemper Vaccine [CDV], and dogs in 'test group' were switched to diet supplemented with spray dried BC. Response to CDV vaccine was evaluated by measuring vaccine specific plasma IgG. Gut Associated Lymphoid Tissue [GALT] response was assessed by measuring fecal IgA. Gut microflora was evaluated by Temporal Temperature Gel Electrophoresis (TTGE) methodology. Repeated measures analysis of variance was used to test for differences between groups and statistical significance considered to be $p < 0.05$.

Dogs fed diets supplemented with BC demonstrated enhanced immune status by showing significantly higher vaccine response and significantly higher levels of fecal IgA as compared to the control group. Supplementing diets with BC also resulted in significantly increased gut microflora diversity and stability [both measures of enhanced gut health] in the test group. In conclusion, diets supplemented with BC significantly enhanced immune health and gut health in dogs.

## ABSTRACT NM-2

**EFFECTS OF WEIGHT LOSS IN OBESE DOGS ON A RANGE OF RENAL BIOMARKERS.** A Tvarijonaviciute[1], JJ Ceron[1], SL Holden[2], PJ Morris[3], V Biourge[4], AJ German[2]. [1]Department of Animal Medicine and Surgery, Veterinary School, University of Murcia, Spain, [2]Department of Obesity and Endocrinology, University of Liverpool, UK, [3]WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, UK, [4]Royal Canin Research Centre, Aimargues, France

Human obesity is a risk factor for development and progression of CKD, and weight loss can reverse the associated manifestations. To date, information is limited regarding the potential for altered renal function in canine obesity. The aim of the current study was to measure a range of renal biomarkers in obese dogs, both before and after weight loss.

Thirty-seven overweight client-owned dogs undergoing weight loss were studied, representing a range of breeds, ages and genders. Both before and after weight loss, body composition was measured by dual-energy X-ray absorptiometry, and plasma concentrations of urea, creatinine, homocysteine, cystatin C, and clusterin were measured using validated assays. Urinalysis was also performed, which included measurement of specific gravity (by refractometer) and protein:creatinine ratio (UPCR).

Urea ($P = 0.043$) and urine specific gravity ($P = 0.012$) increased after weight loss, whilst creatinine decreased ($P = 0.026$). Homocysteine ($P < 0.001$), cystatin ($P < 0.001$) and clusterin ($P < 0.001$) also decreased with weight loss. UPCR was more commonly abnormal pre-weight loss than post-weight loss (8/27 vs. 1/27; $P = 0.015$). On multivariable linear regression analysis, change in lean mass was associated with percentage weight loss (greater weight loss, more lean tissue loss; $R = -0.67$, $R^2 = 0.45$, $P < 0.001$) and pre-weight-loss plasma clusterin concentration (greater clusterin, more lean tissue loss; $R = 0.48$, $R^2 = 0.23$, $P = 0.003$).

These results suggest possible subclinical alterations in renal function in canine obesity, which improve with weight loss. Further work is required to determine the nature of these alterations and, most notably, the reason for the association between pre-weight loss plasma clusterin and subsequent lean tissue loss during weight management.

## ABSTRACT NM-3

**MRNA EXPRESSION RESPONSE OF ADIPOKINES TO FELINE OBESITY DEPENDS ON ADIPOSE TISSUE LOCATION.** H Van deVelde[a], GPJ Janssens[a], H de Rooster[a], I Polis[a], K Piron[a], I Peters[b], A Verbrugghe[a], M Hesta[a]. [a]Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, [b]School of Veterinary Science, University of Bristol, Bristol, United Kingdom

Obesity is an emerging problem in many species, including companion animals. Concomitant disorders such as insulin resistance, diabetes mellitus and dyslipidemia are directly or indirectly linked with an increase of adipose tissue (AT). In humans and rodents obesity is associated with enlarged adipocyte cell size, subsequently creating hypoxia in AT. This altered perfusion can result in necrosis whereby macrophages and lymphocytes are attracted. In contrast to the situation in humans and rodents where data on inflammation in AT -explaining the co-morbidities in obesity- exist, this information is lacking in cats. Therefore, AT mRNA expression of chemokines and adipokines was measured in chronically obese cats. The aim of the present study was to increase the knowledge of the development of obesity and related disorders in cats.

Ten chronically obese cats with a mean body weight (BW) of $6.2 \pm 0.4$ kg and a body condition score (BCS) of $8 \pm 0.2$ on a 9-point scale were included in this study. Ten lean cats with a mean BW of $3.9 \pm 0.2$ kg and BCS of $5 \pm 0.1$ were included as control group. Under general anesthesia, samples were taken at five different fat depots, namely subcutaneous (SC) abdominal AT, SC inguinal AT, bladder AT, renal AT and omental AT. mRNA expression was determined in the AT using a two-step quantitative real time RT-PCR for leptin, adiponectin, IL-6, IL-10, TNF-$\alpha$, IFN-$\gamma$, IL-8, MCP-1 (a chemokine for monocytes) and RANTES (a chemokine for T-lymphocytes). A relative copy number value was calculated for each sample with the results normalised to the three stably expressed housekeeper genes (HPRT1, YWAZ and RPS7). Statistical analyses were performed using a one-way ANOVA for which significance was set at $P < 0.05$.

Body weight and BCS was significantly higher in obese cats compared to lean cats ($P < 0.001$). Obese cats showed higher leptin mRNA expression in the five fat depots ($P < 0.01$, SC inguinal AT $P = 0.039$), with lower adiponectin mRNA expression in bladder ($P = 0.017$) and renal AT ($P = 0.047$), compared to lean cats. In SC inguinal AT, mRNA expression of obese cats was higher for IFN-$\gamma$ ($P = 0.045$), MCP-1 ($P = 0.03$) and RANTES ($P = 0.004$) than in lean cats. At other AT locations, higher expression of inflammatory cytokines was observed in obese cats compared to lean cats, but they failed to reach the level of significance.

This study indicates that the response of feline AT to obesity is present and differs according to its location. Expression in SC inguinal AT showed most changes, suggesting that this AT location might best reflect the changes in inflammatory and immune status of the obese cat. The increase of RANTES and MCP-1 illustrates the attraction of lymphocytes and monocytes into the AT, which suggests a local inflammation of this latter tissue during feline obesity.

## ABSTRACT NM-4

**COMPARISON OF TWO WEIGHT LOSS DIETS IN OVERWEIGHT CATS.** I Jeusette[1], A Tvarijonaviciute[2], J Ceron[2], A Salas[1], N Sanchez[1], M Compagnucci[1], S Fernandez[3], Ll Vilaseca[1], C Torre[1]. [1]Affinity-Petcare, Barcelona, Spain, [2]Veterinary Faculty, University of Murcia, Murcia, Spain, [3]DOC'S veterinary clinic- Premià, Barcelona, Spain

Inflammatory and oxidative pathways are involved in the pathophysiology of feline obesity. Dietary supplementation with olive oil or flavanones induces lower oxidative stress and better inflammatory status in overweight cats. High-protein low-starch diets improve body composition and insulin sensitivity in obese cats, however, they have been suggested to affect negatively bone or renal status. The purpose of this study was to compare 2 diets for body weight loss (BWL) in cats. The hypothesis was that diet A [*as is*, measured metabolisable energy (ME) 3290kcal/kg, protein 40%, fat 11%, starch 17%, total dietary fiber (TDF) 22%, calcium (Ca) 1.5%, phosphorus (P) 0.9%, omega-3 fatty acids 2.3%, 1% fish oil, 0.5% olive oil, 0.06% flavanoids] would induce a safe BWL with improved body composition, glycemic control, inflammatory status and oxidative stress, compared to diet B [*as is*, ME 3310 kcal/kg, protein 36%, fat 8%, starch 28%, TDF 18%, Ca 1.9%, P 1.0%, omega-3 fatty acids 0.15%] used as a regular obesity control diet.

Healthy colony cats ($n = 11$) with body condition score $> 7/9$ were fed a maintenance diet until T0. Then, 6 cats were fed diet A and 5 cats diet B to induce a BWL between 1 and 2% of starting BW per week. Fasting blood and urine were obtained at T0,

after 3 weeks and after 3 months of energy restriction. Complete blood count, serum biochemistry profile, serum fructosamine, amylase, haptoglobin, paraoxonase type 1 (PON-1, an antioxidant and anti-inflammatory enzyme), IGF-1, butyrylcholinesterase activity, urine analysis (protein, creatinine, albumin, fractional excretion of ions), body composition (deuterium dilution), markers of bone formation (serum bone alkaline phosphatase, BAP) and resorption [serum carboxy-terminal telopeptide fragment (CTX), urinary total deoxypyridinoline (DPD)] and ultrasound of the abdomen were assessed. To evaluate any difference between diets, differences from baseline T0 were compared (univariate ANOVA). To assess diet and BWL effects, multivariate ANOVA for repeated measurements was performed (diet, time, diet*time effects). Values are considered as statistically different for P value $< 0.05$.

No difference between diets was observed for energy intake and BWL. Diet A resulted in significantly better lean mass preservation, higher decrease in fructosamine and haptoglobin and in higher increase in PON1 than diet B. Both diets significantly decreased basal insulin, amylase, butyrylcholinesterase, alkaline phosphatase and BAP concentrations, and increased urinary Ca excretion and serum Ca with time**.**

In conclusion, both diets resulted in a safe and similar BWL. However, diet A showed additional benefits for lean mass preservation, glucose control (fructosamine), oxidative and inflammatory status (haptoglobin, PON1), without side effects for renal health (BUN, creatinine, urinary albumin) and liver health (liver enzymes, ultrasound). A decreased marker of bone formation with both diets at T3, with a trend for increased resorption marker (DPD), requires further research.

## ABSTRACT NM-5

**COMPARISION OF HIGH FIBER AND LOW CARBOHYDRATE DIETS ON OWNER-PERCEIVED SATIETY OF CATS DURING WEIGHT LOSS.** AL Witzel[1], TD Moyers[1], MG Cline[1]. [1]University of Tennessee Department of Small Animal Clinical Sciences, Knoxville, TN

Food seeking behaviors exhibited by cats during weight loss programs are frustrating to owners. Two categories of prescription weight loss diets are currently available for cats, high fiber (HF) and low carbohydrate (LC). This study's goal was to determine if cat owners perceive a difference in satiety when cats are fed either a HF or LC diet during weight loss. 28 client-owned cats were randomly assigned to either a HF (protein 12.3g, carbohydrate 10.2g, fiber 5.0g/100kcal) or LC (protein 11.1g, carbohydrate 1.0g, fiber 0.6g/100kcal) canned diet and fed to 80% of their ideal weight resting energy requirements. Cats were rechecked at 2, 4, 6, and 8 weeks and food intake adjusted to maintain weight loss between 0.5-2%/week. Owners completed behavior questionnaires at each visit and were blinded to food assignments. A logistic regression model using a backward selection procedure was used to examine the effect of diet, adjusted for covariables on the owner' response to each question. The two diet groups did not differ by age, sex, body condition score, or rate of weight loss. LC cats consumed more calories (210kcal/day) than HF cats (184kcal/day) on average ($p < .05$). HF cats were less likely to pace (23% vs. 46%, $p = .01$) or beg (42% vs. 62%, $p = .02$) at the food bowl. Cats fed the LC diet were more likely to forage or steal food (61% vs. 43%, $p = .06$). Owners feeding the HF diet were more satisfied with their cats' weight loss programs than those on the LC diet ($p = .006$). In conclusion, HF diets may be superior to LC diets for minimizing food-seeking behavior and improving owner satisfaction during weight loss.

## ABSTRACT NM-6

**SEASON AND MONTH EFFECTS ON FOOD INTAKE IN ADULT COLONY CATS.** S Serisier[1], A Feugier[1], C Venet[1], Y Soulard[1], V Biourge[1], AJ German[2]. [1]Royal Canin Research Center, Aimargues, France, [2]Department of Obesity and Endocrinology, Institute of Aging and Chronic Disease, University of Liverpool, Liverpool, United Kingdom

There are numerous reports about seasonal cycles on food intake in animals but information is limited in dogs and cats.

During the course of a 6-year retrospective study (from 2004 to 2009), food intake was recorded in 38 *ad-libitum*-fed adult colony cats. These cats were involved in palatability studies and, therefore, the whole group was fed a different dry diet each day. Individual food intake was recorded on a daily basis, and the mean daily intake for each calendar month was calculated. Among these 38 cats, 17 were male (15 neutered) and 21 were female (10 neutered), whilst, 32 were pure breeds and 6 were mixed breeds. At study completion, median (range) age was 8.2 years (y) (7.4-9.6y); 22 cats were in ideal condition (BCS = 5/9), whilst 16 cats were overweight (BCS > 5/9). Cats were housed in closed indoor/outdoor runs with 30 having unlimited outdoor access, and the remaining 8 housed indoor. Housing and management protocols adhered to European regulatory rules for animal welfare. The mixed procedure of SAS© was used. The statistical model included either season and year or month and year with their respective interactions as fixed effect and cat was defined as a random term.

Irrespective of year, a seasonal effect was evident ($p < 0.001$), with food intake during spring and summer being less than food intake during autumn and winter. Further, again irrespective of year, a monthly effect was identified ($p < 0.001$) with food intake being greatest in January, February, October, November and December; intermediate in March, April, May and September; and least in June, July and August (Table 1).

**Abstract NM-6:**

**Table 1.** Least square means of food intake per month of adult colony cats from 2004 to 2009.

| Month | Jan | Feb | Mar | Apr | May | June | July | Aug | Sept | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Food intake (g) | 57.2 | 56.3 | 54.8 | 52.8 | 53.2 | 52.0 | 50.4 | 51.5 | 54.5 | 57.5 | 58.0 | 57.9 |
| SEM | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |

In conclusion, whatever the year, average food intake in July is 15% less than food intake in December. This variation of food intake could be the result of variation of outside temperatures, differences in daylight length, and/or haircoat changes. This seasonal effect in food intake should be properly considered when estimating daily maintenance energy requirements in cats.

## ABSTRACT NM-7

**INFLUENCE OF DIFFERENT SOURCES OF CORN STARCH ON STOOL CHARACTERISTICS IN DOGS OF DIFFERING BODY SIZES.** R Goudez[1],2, M Weber[2], V Biourge[2], JS Suchodolski[3], L Martin[1], V Leray[1], JM Steiner[3], H Dumon[1], P Nguyen[1]. [1]LUNAM University, Oniris, National College of Veterinary Medicine, Food and Science and Engineering, Nutrition and Endocrinology Unit, Nantes, France, [2]Royal Canin Research Center, Aimargues, France, [3]GI Laboratory, Texas A&M University, College Station, Texas

Previous works from our laboratory showed that large dogs produce poorer quality stools compared to small dogs. This difference could be related to a higher fermentative activity in the hindgut of large dogs and could be modulated by dietary protein and fiber sources. The aim of the present study was to assess the effect of different corn starch sources on microbial communities and fecal characteristics in large and small breed dogs.

Five female miniature schnauzers (MS, 6.5-8.5 kg) and 5 female German shepherds (GS, 24.7-33.5 kg) were included in the study. Three iso-formulated extruded diets (37.9±1.1% starch, 25.2±0.4% protein, 14.8±0.3% fat, 7.9±1.9% TDF, 6.2±0.3% ash, 3834 kcal ME/Kg, as fed basis) were tested in

a crossover design. Diets contained the same protein sources but varied in the source of corn starch (flour, purified, and high-amylose). Fresh feces were collected and their consistency was scored daily, from 1 (hard) to 5 (liquid). Fecal volume, moisture, pH, lactate and short chain fatty acid [SFCA] concentrations were measured. Fecal microbial communities were characterized by 454-pyrosequencing of the 16S rRNA gene. Effects of diet or breed on fecal parameters were statistically tested using Friedman and Kruskal-Wallis tests. The Unifrac metric followed by analysis of similarity (ANOSIM) was used to assess differences in microbial communities among diets and breeds.

Regardless of diet, a breed effect was found for all fecal parameters ($p < 0.017$) and microbial communities ($p < 0.001$). *Lactobacillaceae* were significantly higher in fecal samples of GS compared to MS. Unifrac analysis revealed significant differences in microbial communities between both breeds. In GS, corn starch sources had a significant effect on fecal consistency and lactate concentrations ($p < 0.007$), as well as on pH, fecal volume, and SCFA concentrations ($p < 0.022$). MS appeared more tolerant and only fecal volume ($p < 0.015$) and lactate concentrations ($p < 0.022$) were affected. Dogs on the high-amylose diet had the lower fecal pH, and higher SCFA and lactate concentrations, and increased fecal volume. In contrast, dogs on the purified starch diet had stools with a harder consistency, a decreased fecal volume and decreased concentrations of fermentation products. The sources of corn starch had an impact on microbial communities in both breeds ($p < 0.035$). *Firmicutes* were lower on the corn flour starch diet [64.30% (53/90)] compared to the purified and high-amylose starch diets [93.78% (91/96) and 96.48% (87/98)]. This change was associated with a decrease in *Fusobacteria* and *Bacteroidetes*.

These results suggest that the source of corn starch has an impact on fecal consistency, fecal microbial communities, and fermentation products. These effects of the starch sources were not consistent and may be due to differences in fermentative activities as well as microbial communities between dog breeds.

## ABSTRACT NM-8

## A 8.5-YEAR LONGITUDINAL STUDY TO IDENTIFY RISK FACTORS OF OBESITY IN COLONY CATS.

S Serisier[1], A Feugier[1], C Venet[1], Y Soulard[1], V Biourge[1], AJ German[2]. [1]Royal Canin Research Center, Aimargues, France, [2]Department of Obesity and Endocrinology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom

Several transverse studies have assessed factors predisposing cats to becoming overweight. Generally, demographic factors (e.g. breed, age, gender, sex status, housing), activity and diet have been examined in adult cats, but early-life factors have not been considered. In humans, studies have demonstrated that the risk of being overweight in adulthood is largely determined early in childhood. The aim of this retrospective cohort study was to identify risk factors for the development of overweight in colony cats fed dry diets *ad libitum*.

A total of 80 colony-cats were studied, representing 14 different breeds, with 36 males (34 neutered) and 44 females (24 neutered). Of these cats, 36 were determined to be overweight (OW; body condition score [BCS] > 5/9), whilst the remaining 44 were in ideal condition (I; BCS = 5/9). The effects of various factors (including age, gender, neuter status, breed [pure vs. mixed], mean daily food intake [measured over a 12-month period], housing status [indoor with outdoor access vs. exclusively indoor] and body weight [BW] at 1year [y] of age [BW1y; BCS was 5/9 in all cats at this age]) on weight status was assessed. Risk factors for being overweight as an adult were examined using PLS-DA analysis (Tanagra©).

The main significant descriptor variables that predicted the likelihood of being overweight were food intake (median 46.3g [range: 32.8-73.4g] vs. 62.7g [38.1-96.6g]) and BW1y (3.02 kg [1.76-5.46kg] vs. 3.95 kg [2.22-6.50kg]). A statistical model was then created (mixed procedure of SAS©; group [OW vs I] included as a fixed effect and cat as a random term) to assess changes in BW and food intake between 1y and 8.5y of age where data were available (42/80 cats [25 I; 17 OW]). Differences between groups (OW vs I) were noted for both BW ($p < 0.001$) and food intake ($p = 0.002$). BW increased significantly with age only in the OW group ($p < 0.001$), whilst food intake did not change with age in either group. Interestingly, when BW1y was included as a covariate in the model, no differences in either BW ($p = 0.17$) or food intake ($p = 0.07$) were noted, suggesting that most effects could be explained by BW1y. Finally, given the importance of BW1y, changes in BW from 3 months (mo) to 1y were assessed where data were available (16/80 cats [8I; 8OW]; SAS PROC MIXED analysis with BW3mo included as a covariate). At 3 mo of age, no difference in BW was observed between the 2 groups. However, BW diverged in the two groups tending to become different at 1y of age ($p = 0.1$).

In conclusion, BW at 1 year of age is suggested to be a major risk factor for cats becoming overweight. Further investigations are necessary to understand which factors (e.g. genetics, rate of growth, food intake, physical activity) may be responsible for BW differences at this age. Nonetheless, identifying at-risk cats at 12 months, even before the onset of obesity, could enable strategies aimed at preventing feline obesity to be better targeted.

## ABSTRACT NM-9

## BALL FEEDING INCREASES PHYSICAL ACTIVITY AND REDUCES BODY WEIGHT REGAIN AFTER A WEIGHT LOSS DIET IN CATS.

A Salas, I Jeusette, M Compagnucci, Ll Vilaseca, C Torre. Affinity Petcare, Barcelona, Spain

After a weight loss program, cats have difficulties to maintain body weight (BW) and usually they regain their initial BW quicker than desired. A lack of owner compliance and a decrease in energy expenditure are the main causes for this quick regain. Feeding enrichment with puzzle feeders, from which food has to be obtained by rolling, have been proposed to help obese cats to lose BW while improving physical welfare. The purpose of this study was to evaluate if enrichment with a feeding ball would increase objectively-measured physical activity, food intake and its repercussion on BW after a weight loss diet.

Eleven healthy colony cats were used for this study. Cats had been firstly energy-restricted for 3 months to induce a 15% BW loss with 2 different low-fat energy-restricted diets [diet A ($n = 6$), *as is*, measured metabolic energy (ME) 3290 kcal/kg, protein 40%, fat 11%, starch 17%, total dietary fiber (TDF) 22%; diet B ($n = 5$), *as is*, ME 3310 kcal/kg, protein 36%, fat 8%, starch 28%, TDF 18%]. Then, in a cross-over design, each group of cats received during 3 months their low-fat diet *ad libitum* in 2 different feeding conditions: in a conventional bowl (CB) or in a feeding ball (FB). Daily physical activity was monitored by accelerometers that cats wore in the harness. Daily food intake and weekly body weight were recorded. A general linear model (GLM) with repeated measures was performed to assess feeding condition, period and diet effects on physical activity, BW regain and food intake. Values were considered as statistically significant for P value $< 0.05$.

As there was no difference between diets, data from both diets were pooled and only feeding condition and period effects are discussed. A significant effect of the cross-over period was observed on food intake and BW regain independently of the type of feeding, during the first 3-months period, food intake was higher (55±7 kcal/kg BW vs 46±7 kcal/kg BW; $p = 0.000$) and BW regain was faster (0.8%/week vs 0.09%/week; $p = 0.000$) than in the second period in both diet groups, probably due to a seasonal effect and/or a compensatory effect after energy restriction. Feeding *ad libitum* with a FB versus CB resulted in an increased physical activity (+34%, $p = 0.000$), in a reduced food intake (-3.74 kcal/kg BW, $p = 0.000$) and in a lower BW regain of the cats (3.8±2.2% vs 7.6±2.5%, $p = 0.014$).

In conclusion, feeding with FB could be an interesting strategy to apply in overweight cats after weight loss programs to increase physical activity, reduce food intake and consequently slow down BW regain or simply to promote physical activity and consequently improve the wellbeing of normal or overweight cats.

**Abstract NM-1:**
**Table 1.** Mean abundance ± standard deviation (M ± SD) of bacterial DNA for each bacterial group at the various treatment levels.

| | Treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | 0.2% inulin | | 0.4% inulin | | 0.2% scFOS | | 0.4% scFOS | |
| | M±SD | M±SD | p | M±SD | p | M±SD | p | M±SD | p |
| Ruminococcaceae | 6.6±0.5 | 6.9±0.1 | 0.68 | 6.5±0.3 | 1.00 | 6.7±0.2 | 0.96 | 6.6±0.2 | 1.00 |
| *Faecalibacterium*spp. | 4.1±0.8 | 5.0±0.2 | 0.22 | 4.6±0.9 | 0.42 | 4.8±0.5 | 0.16 | 4.9±0.4 | 0.09 |
| Turicibacterspp. | 6.6±0.6 | 7.1±0.4 | 0.77 | 6.6±0.7 | 1.00 | 6.9±0.3 | 0.85 | 7.0±0.6 | 0.75 |
| *Bifidobacterium*spp. | 2.9±1.2 | 3.5±1.4 | 0.81 | 2.7±1.2 | 0.99 | 2.6±0.7 | 0.96 | 3.3±1.4 | 0.72 |
| *Lactobacillus*spp. | 3.9±1.3 | 4.0±1.4 | 1.00 | 4.2±1.0 | 0.99 | 4.7±1.5 | 0.72 | 4.6±1.9 | 0.81 |
| Bacteroidetes | 4.6±1.0 | 5.1±0.1 | 0.78 | 5.1±0.4 | 0.54 | 4.8±0.7 | 0.94 | 5.2±0.5 | 0.36 |

## ABSTRACT NM-10

**PLASMA ADIPOKINE CONCENTRATIONS IN LEAN AND OBESE DOGS.** N Berghoff[1], R Stone[2], JS Suchodolski[1], JM Steiner[1], DL Zoran[3]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, [2]North Houston Veterinary Specialists, Humble, TX, [3]Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX

White adipose tissue does not only provide energy storage, but also produces inflammatory cytokines, as well as adipokines necessary for normal metabolism. In humans, the release of these mediators is believed to lead to a state of chronic low-grade inflammation, which may play a role in the development of insulin resistance and diabetes mellitus. Studies investigating plasma cytokine and adipokine concentrations in dogs with naturally occurring obesity are lacking. Thus, the aim of this study was to measure plasma cytokine and adipokine concentrations in client-owned lean and obese dogs, and to determine if a sub-inflammatory state is present in obese dogs.

Blood samples were collected from 24 healthy lean dogs (body condition score [BCS]: 4-5 out of 9) and 19 otherwise healthy obese dogs (BCS: 7-9 out of 9). Plasma concentrations of cytokines (GMCSF, IFN-γ, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, MCP-1, TNF-α), leptin, adiponectin, resistin, and insulin were measured using a multiplex bead antibody assay system (Millipore, MA). C-reactive protein (CRP) concentrations were measured by ELISA (Tridelta, NJ). Complete blood count (CBC) and plasma biochemical analyses were carried out using routine automated analyzers. Test results were compared between lean and obese dogs using an unpaired Student's t-test or Mann-Whitney U test, as appropriate. Statistical significance was set at $p < 0.05$.

Plasma leptin and insulin concentrations were significantly higher in obese dogs than in lean dogs ($p < 0.0001$ and $p = 0.0324$, respectively). No differences were found for adiponectin, resistin, any of the cytokines, or CRP concentrations between the two groups. White blood cell and absolute neutrophil counts were significantly higher in obese than in lean dogs ($p = 0.0379$ and $p = 0.0073$, respectively). Obese dogs had significantly higher plasma cholesterol ($p = 0.0192$), total protein ($p = 0.0035$), globulin ($p = 0.0127$), and triglyceride ($p = 0.0022$) concentrations than lean dogs, whereas the creatinine concentration was lower in obese dogs than in lean dogs ($p = 0.0009$).

Obese dogs in this study had high plasma leptin concentrations, which is characteristic for obesity. Interestingly, the plasma adiponectin concentration was not different between lean and obese dogs. Reduced plasma adiponectin concentrations have been reported in obese humans and cats, while conflicting reports exist for dogs. Low adiponectin concentrations were reported in dogs with experimentally induced obesity, but not in dogs with naturally occurring obesity, possibly suggesting metabolic differences. The increase in WBC and neutrophil counts in obese dogs suggests a possible low-grade inflammation in these dogs, even though no differences in plasma cytokine and CRP concentrations were found between the two groups. It is possible that the cytokine markers used in this study were not able to reliably detect differences in plasma concentrations, or that plasma cytokine measurements are not optimal for assessing the overall inflammatory state in dogs.

## ABSTRACT NM-11

**EFFECT OF LOW LEVEL FRUCTAN SUPPLEMENTATION ON THE ABUNDANCE OF SPECIFIC BACTERIAL GROUPS IN FECAL SAMPLES OF DOGS.** Y Minamoto[1], KA Barry[2], JF Garcia-Mazcorro[1], ME Markel[1], NM Gossett[1], GC Fahey Jr[2], JM Steiner[1], KS Swanson[2], JS Suchodolski[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, [2]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL

Recent metagenomic studies in dogs with gastrointestinal disease have shown a decrease in specific bacterial groups such as Ruminococcaceae, *Faecalibacterium* spp., *Turicibacter* spp., and Bacteroidetes. Fructans are considered to have a prebiotic effect and have the potential to modulate the intestinal microbiota. The aim of this study was to evaluate the effect of low level fructan supplementation on the abundance of those bacterial groups that are frequently found to be decreased in dogs with gastrointestinal disease.

Leftover frozen fecal samples from five research dogs utilized in a previous low level fructan supplementation study were analyzed. Briefly, five diets that contained either cellulose alone or that were supplemented with either inulin or short-chain fructooligosaccharides (scFOS) each at 0.2% or 0.4% were evaluated in a 5 × 5 Latin square design. Bacterial DNA was extracted from each fecal sample and evaluated for the abundance of Ruminococcaceae, *Faecalibacterium* spp., *Turicibacter* spp., *Bifidobacterium* spp., *Lactobacillus* spp., and Bacteroidetes using quantitative PCR assays. For statistical analysis, a general linear mixed model, adjusted for Tukey post-test comparisons, was used to compare the abundance of bacterial groups in response to the different diets. Significance was set at $p < 0.05$.

No significant differences in the abundance of the evaluated bacterial groups were observed in response to inulin or scFOS (Table 1). There was a trend for *Faecalibacterium* spp., to increase with supplementation of 0.4% scFOS, but this increase did not reach significance ($p = 0.09$).

In conclusion, in this study low level fructan supplementation did not significantly alter the abundance of selected bacterial groups in healthy adult research dogs. Further studies are needed to evaluate the effect on the abundance of these bacterial groups at higher levels of fructan supplementation.

## ABSTRACT NM-12

**SOY GERM ISOFLAVONES SUPPLEMENTATION REDUCED BODY FAT ACCUMULATION AND ENHANCED ENERGY METABOLISM IN DOGS.** Y Pan. Nestlé Purina Research, Checkerboard Square, St. Louis, MO

Up to 40% dogs in developed countries are either overweight or obese. Obesity increases the incidence of many chronic diseases in dogs. One of the known risk factors for obesity in dogs is spaying/neutering which results in significant decline in energy metabolism due to loss of sex hormones. So reversing the decline in energy metabolism by natural estrogen analogs will be very effective in reducing the risk of obesity in spayed/neutered dogs by maintaining healthy energy metabolism. Published research shows that purified daidzein and glycitein were able to mimic estrogen to prevent body fat accumulation.

Since the isoflavones in soygerm meal are mainly daidzein and glycitein, we investigated the effects of isoflavones from soybean germ meal (SGM) on body fat accumulation, and energy metabolism in spayed/neutered dogs with normal body weight. Based on baseline maintenance energy requirement (MER), body weight, and % body fat, spayed/neutered Labrador Retrievers with normal body weight were randomized into two groups: control, and isoflavones. Both the control and isoflavone diets had comparable levels of metabolizable energy, protein, fat, carbohydrates, and minerals. The dogs were allowed to ingest 25% more than their baseline MERs for 9 months. Body composition using DEXA, thyroid function, complete blood count, and blood clinical chemistry parameters were measured at the baseline and every three months after initiation of the feeding trial. Total energy expenditure was measured using doubly-labeled water protocol at the end of the study. The results show that the isoflavones from SGM significantly increased daily energy expenditure and reduced body fat accumulation in dogs without reduction in food intake.

# SMALL ANIMAL – ONCOLOGY

**ABSTRACT O-1**
**CHARACTERIZATION OF BETA-CATENIN EXPRESSION IN CANINE ORAL MELANOMA.** E Chon, V Thompson, M Huelsmeyer, T Stein. University of Wisconsin – Madison, Madison, WI

Canine oral melanoma is a highly aggressive malignancy associated with poor survival. Perturbation of the Wnt/beta-catenin signaling pathway has been implicated in the progression of human melanoma. However, there currently is no information regarding this pathway's role in canine oral melanoma. Our objective was to characterize beta-catenin expression in canine oral melanoma tumors and cell lines.

Initial characterization of beta-catenin expression was performed with immunohistochemistry of twelve archived canine oral melanoma tumors. For further characterization, we utilized nine cell lines derived from spontaneously arising canine oral melanomas. Cell lysates from each cell line was fractionated and beta-catenin protein expression was semi-quantitatively determined with Western Blot analysis. A human melanoma cell line having a known stabilizing mutation in beta-catenin served as a positive control.

Among the twelve histologic samples, nine stained positive for beta-catenin. Differential intracellular staining (cytoplasmic vs. nuclear vs. membranous staining) was evident in positively stained tumors. Similarly, subcellular fractionation of cell line lysates revealed differential staining between cell lines and within subcellular fractions.

Differential expression of beta-catenin suggests the Wnt/beta-catenin signaling pathway may be active in a subset of canine oral melanomas. (VCS Award Winner)

**ABSTRACT O-2**
**AGREEMENT BETWEEN FLOW CYTOMETRY, PCR FOR ANTIGEN RECEPTOR REARRANGEMENT, AND IMMUNOHISTOCHEMISTRY IN CANINE LYMPHOMA.** LB Thalheim, SE Suter, LB Borst, LE Williams. North Carolina State University College of Veterinary Medicine, Raleigh, NC

Immunohistochemistry (IHC), flow cytometry (FC), and PCR for antigen receptor rearrangement (PARR) are utilized to help diagnose ambiguous cases of canine lymphoma and to determine immunophenotype. Objectives of this study were to calculate level of agreement between IHC, FC, and PARR in canine lymphoma and to determine the ability of FC and PARR to correctly predict immunophenotype as defined by IHC.

Medical records were searched to identify dogs with lymphoma that had concurrent FC, PARR and IHC performed. Fifty-two cases were identified. Immunophenotype results were categorized as T-cell, B-cell, dual T-/B-cell, and negative. The percent agreement between tests was calculated. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of FC and PARR in correctly determining immunophenotype were also calculated.

The percent agreement between FC and IHC was 94.2%, between PARR and IHC was 65.4%, and between all three tests was 59.6%. For B-cell cases, the sensitivity, specificity, PPV, and NPV for FC were 92%, 100%, 100%, and 81% respectively, and for PARR were 62%, 100%, 100%, and 46% respectively. For T-cell cases, the sensitivity, specificity, PPV, and NPV for FC were 100%, 97%, 93%, and 100% respectively, and for PARR were 77%, 97%, 91%, and 93% respectively.

The percent agreement between FC and IHC was significantly higher compared to PARR and IHC. While the PPVs are similar between FC and PARR, the overall sensitivity of PARR in detecting correct phenotype is lower compared to FC. Based on these results, FC is superior to PARR in determining immunophenotype. (VCS Award Winner)

**ABSTRACT O-3**
**FIROCOXIB HAS ANTITUMOR ACTIVITY AS A SINGLE AGENT AND ENHANCES THE ACTIVITY OF CISPLATIN IN DOGS WITH TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER.** DW Knapp[1], CJ Henry[2], WR Widmer[1], KM Tan[1], GE Moore[1], JA Ramos-Vara[1], MD Lucroy[1], CB Greenberg[1], SN Greene[1], AH Abbo[1], PD Hanson[3], R Alva[3], PL Bonney[1]. [1]From Purdue University, West Lafayette, IN;, [2]University of Missouri, Columbia, MO; and [3]Merial Limited, Duluth, GA

Cisplatin combined with a nonselective cyclooxygenase (cox) inhibitor has potent antitumor activity against transitional cell carcinoma (TCC) of the urinary bladder in dogs, but this combination treatment is limited by renal toxicosis. Cox-2 is expressed in TCC, but in limited sites within the kidney, suggesting that a cox-2 inhibitor may be safely combined with cisplatin. The hypothesis was that cisplatin combined with a cox-2 inhibitor would have greater antitumor activity, but no more renal toxicity, than cisplatin alone in dogs with TCC. A randomized clinical trial was performed at Purdue University and the University of Missouri. Entry requirements included dogs with histopathologically-confirmed TCC of the urinary bladder, measurable lesions in the bladder, normal serum creatinine, no prior cisplatin treatment, expected survival of at least 6 weeks, and informed pet owner consent. Dogs were excluded if they had received a cox inhibitor within the previous 14 days, or received one for more than a month in the previous 3 months.

Dogs were randomized to receive cisplatin (60 mg/m$^2$ IV q21d with saline diuresis), firocoxib (Previcox®, Merial, 5 mg/kg PO q24h), or the combination. Complete evaluation including tumor staging and measurements was performed before, and at 6-week intervals during therapy. Renal function was monitored by serum creatinine concentration, iohexol clearance, and urine specific gravity. Toxicoses were graded according to Veterinary Co-Operative Oncology Group criteria.

The primary endpoint was remission rate. It was expected that 34 dogs could be needed in each treatment group to detect a 35% difference in remission rate (power 0.90; two-sided test). Interim analyses were scheduled to be done after entering 12 and 25 dogs per group. At the first interim analysis, the remission rate (all partial remissions) with cisplatin/firocoxib (8 of 14 dogs, 57%) was significantly ($P = 0.021$) greater than that with cisplatin alone (2 of 15 dogs, 13%). The progression free interval was longer ($P = 0.026$) with cisplatin/firocoxib (median 186 days) than with cisplatin alone (median 87 days). Renal and gastrointestinal toxicoses were common in dogs receiving cisplatin, but there were no significant differences between dogs receiving cisplatin or cisplatin/firocoxib. Firocoxib alone induced remission or stable disease in 3 of 15 (20%) and 5 of 15 (33%) of dogs, respectively. In conclusion, firocoxib significantly enhanced the antitumor activity of cisplatin resulting in a remission rate of 57%. Although firocoxib did not enhance the toxicity of cisplatin, the toxicoses inherent to cisplatin (even as a single agent) may affect the decision as to whether to use this treatment or not. Firocoxib had antitumor activity and minimal side effects as a single agent, and can be considered a palliative treatment for dogs with TCC.

**ABSTRACT O-4**

**PHASE II CLINICAL TRIAL OF VINORELBINE AND PIROXICAM FOR DOGS WITH TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER.** ME Kaye[1], JA Lawrence[1], K Weishaar[2], DH Thamm[2]. [1]University of Georgia College of Veterinary Medicine, Athens, GA, [2]The Animal Cancer Center, Colorado State University, Fort Collins, CO

Transitional cell carcinoma (TCC) of the urinary bladder in dogs is an invasive, locally aggressive disease with limited effective therapies. Vinorelbine is a semisynthetic vinca alkaloid that has demonstrated activity in advanced human TCC. The purpose of this study was to evaluate the anti-tumor activity and toxicoses of vinorelbine and piroxicam in dogs with TCC.

Dogs were treated with vinorelbine (15 mg/m$^2$ IV) once weekly for four doses followed by every other week until tumor progression. Piroxicam (0.3 mg/kg PO) was administered daily. Tumor response was determined according to Response Evaluation Criteria in Solid Tumors (RECIST); toxicoses were graded according to Veterinary Cooperative Oncology Group (VCOG) criteria. Median time to progression (TTP) and median survival time (MST) were calculated using Kaplan-Meier analysis.

Fourteen dogs were enrolled. A median of 6.5 doses of vinorelbine (1-16) were administered. Two dogs (14%) had partial responses; 8 experienced stable disease for a median of 100 days (56-239d); 2 had progressive disease. Response could not be assessed in 2 dogs. Subjective improvement in clinical signs was noted in 11 dogs. Of 89 total doses of vinorelbine administered, 3 grade IV and 3 grade III neutropenias, 1 grade III thrombocytopenia, and 2 grade III gastrointestinal toxicities were documented. The median TTP was 93 days. The MST from the first vinorelbine treatment was 168 days; MST from initial diagnosis was 432 days.

Vinorelbine and piroxicam appear to have antitumor activity against canine TCC and may be a therapeutic option when managing this disease.

**ABSTRACT O-5**

**COMPUTED TOMOGRAPHIC APPEARANCE OF CANINE THYROID TUMORS.** K Deitz[1], L Gilmour[1], V Wilke[2], E Riedesel[1]. [1]Iowa State University, College of Veterinary Medicine, Ames, IA, [2]University of Minnesota, College of Veterinary Medicine, St. Paul, MN

Computed tomography is often used to help determine the treatment approach to canine thyroid tumors. This study was undertaken to describe the computed tomographic features of canine thyroid tumors.

Nineteen dogs with computed tomographic images of a histopathologically confirmed thyroid tumor were identified. Each CT scan was evaluated by 3 clinicians for various characteristics, independently and then by consensus.

There were 17 carcinomas and 2 adenomas. Tumor consistency was heterogeneous in 16/19 (84%). Most (12/19) had internal mineralization, although in 2 there was extensive bone destruction which made the presence of tumor mineralization difficult to determine. Sixteen tumors (84%) were unilateral. Of these, 11/16 were left-sided, and 5/16 were right-sided. The remaining 3 were considered bilateral or ventral. All tumors had some degree of contrast enhancement. Eighteen had heterogeneous enhancement, with 7/19 having more contrast enhancement peripherally than centrally. Most (16/19) had well-defined margins post-contrast. Location of tumors compared to bony structures was variable, with most having their cranial margin somewhere along the level of the second cervical vertebra (13/19) or at the junction of the second and third cervical vertebra (1/19). Some were much more cranially located, with the cranial margin at the level of the temporomandibular joint (1/19) or bulla (4/19). The caudal extent was variable, and was anywhere from mid C1 to caudal C5.

Common CT features of canine thyroid tumors include unilateral location in the neck anywhere from the temporomandibular joint to C5, with heterogeneous consistency, internal mineralization, and heterogeneous contrast enhancement with well-defined margins.

**ABSTRACT O-6**

**MULTI-INSTITUTION RETROSPECTIVE STUDY OF 85 CASES OF CANINE THYMOMA (1999-2010).** C Robat[1], L Cesario[2], R Gaeta[3], D Schrempp[4], R Chun[4]. [1]ONCOVET (France), [2]Michigan State University, [3]University of Pennsylvania, [4]Purdue University, [5]University of Wisconsin-Madison

Thymomas are uncommon tumors of the thymic epithelium in dogs. The objective of the study was to compile a large number of cases and further characterize the disease in regards to presentation and outcome.

Data was collected retrospectively from 85 cases from 4 institutions over an 11-year period (1999-2010). Signalment, clinical presentation, diagnostic testing, treatment specifics, adverse events, and survival were evaluated.

Eighty-five dogs were included in the study. Labrador Retrievers and Golden Retrievers represented 38 % of the population. The mean age was 9.4 years. The most common presenting signs were cough, lethargy/weakness and tachypnea. Eleven dogs (13%) had clinical signs compatible with myasthenia gravis (7 were confirmed). Total calcium was high in 24/85 dogs (28%) (high ionized calcium in 25/37 dogs). Three dogs had pulmonary metastasis at the time of diagnosis. Surgery was performed in 56/85 patients. Phrenic nerve section occurred in 20% of dogs. Thirteen percent had tumor recurrence (mean 682 days post surgery, median 547d (32-2170)). Thirteen percent of dogs developed a second tumor. For dogs receiving surgery the median survival time was 758 days and without surgery was 103 days. There was no difference in survival time between dogs with and without hypercalcemia or myasthenia gravis at diagnosis.

This study confirms that thymoma is a rare disease with a good prognosis post surgery, even in dogs with a large tumor burden or presenting a paraneoplastic syndrome. An interesting finding is that 13% of dogs developed a second tumor.

**ABSTRACT O-7**

**EVALUATION OF SECRETED FRIZZLED-RELATED PROTEIN 2 EXPRESSION IN CANINE THYROID TUMORS USING RT-PCR AND IMMUNOHISTOCHEMISTRY.** K Deitz[1], K Metivier[2], V Wilke[2], M Ackermann[1], C Wang[1]. [1]Iowa State University, College of Veterinary Medicine, Ames, IA, [2]University of Minnesota, College of Veterinary Medicine, St. Paul, MN

We have previously reported up-regulation of secreted frizzled-related protein 2 (SFRP2) gene expression in canine thyroid tumors using microarray and SFRP2 protein expression and cellular localization were characterized with immunohistochemistry (IHC). The purpose of this study was to validate the gene expression of SFRP2 using RT-PCR. Additional samples were also evaluated via IHC and analyzed statistically with the samples from the initial study to increase statistical power. Tissue samples were obtained from dogs undergoing neck mass removal with informed client consent. Only dogs with thyroid carcinomas confirmed by histopathology were included in the study. Control samples of histologically normal thyroid were obtained from dogs lacking thyroid disease that were euthanized for unrelated reasons. Thyroid tumors from the dogs were categorized based on the degree of invasion: tumor cells confined to the capsule were classified as non-invasive, and those with capsular or vascular invasion were classified as invasive. There were 9 samples in each of the 3 groups (control, non-invasive, and invasive). For RT-PCR, a 1-2cm$^3$ section of tissue was placed in RNA later® and samples were assessed for RNA quality by agarose gel electrophoresis. Only samples with no evidence of degradation were used for further analysis. Beta actin was used as the internal reference gene. The mean expression of SFRP2 mRNA in invasive carcinoma was 288 times higher versus control samples. Mean SFRP2 mRNA expression in non-invasive versus control samples was 50 times higher.

Samples for IHC were placed in formalin and assessed for SFRP2 antigen using goat anti-SFRP2 antibody. There were a total of 35 tumor samples (including 20 from the previous study) and 10 controls (including 4 from the previous study). In all samples, SFRP2 antigen was present in the cytoplasm of neoplastic thyroid follicular epithelial cells. Tumor samples had significantly

higher distribution ($p = 0.000$) and intensity scores ($p = 0.0021$) versus control tissues, which lacked immunoreactivity for SFRP2. This study verifies our previous microarray and IHC results using RT-PCR to assess expression of SFRP2 in thyroid tumors. SFRP-2 is up-regulated in these tumors at all levels and likely plays a role in pathogenesis that requires further elucidation.

**ABSTRACT O-8**

**INVESTIGATING *IN VITRO* ANTI-CANCER EFFECTS OF BENZIMIDAZOLES IN CANINE OSTEOSARCOMA.** JM Schmit, JM Wypij, H Pondenis, TM Fan, LD Garrett. College of Veterinary Medicine, University of Illinois, Urbana, II. USA

Introduction: Benzimidazole (BZ) drugs have demonstrated anti-cancer effects in various tumor models and their direct effects on cell proliferation and apoptosis are hypothesized to be due to microtubule inhibitory actions as well as indirect anti-angiogenic effects via vascular disruption and VEGF modulation. Canine osteosarcoma is a common tumor with an aggressive clinical course and angiogenic phenotype. Despite advances in veterinary medicine, the overall survival has not dramatically improved in recent years, warranting investigation of novel therapies. The aims of this study were to assess effects of the clinically-used benzimidazoles [mebendazole(MBZ), fenbendazole (FBZ), and albendazole (ABZ)] in canine OSA cell lines.

Materials and Methods: Canine osteosarcoma cell lines D17 and HMPOS were incubated for 24-48 hr with varying doses of BZs (ABZ, FBZ, and MBZ, Sigma-Aldrich) based on published pharmacokinetic data and peak plasma concentrations of clinical dosing regimens. Cell proliferation was assessed with a commercial MTS assay (Cell Titer AqueousOne, Promega). Soluble canine VEGF protein was assessed via commercial ELISA (R&D Systems).

Results: In D17 and HMPOS canine OSA cell lines BZs induce a time and dose dependent decrease in cell proliferation and modulate VEGF secretion.

Conclusions: Benzimidazole drugs (albendazole, mebendazole, and febendazole) demonstrate *In vitro* anti-neoplastic effects in canine OSA cell lines. Further investigation is ongoing to evaluate the molecular mechanisms of BZ anti-neoplastic function and the potential for BZs as novel adjuvant treatment for veterinary cancers. (VCS Award Winner)

**ABSTRACT O-9**

**MULTIDRUG RESISTANCE GENE EXPRESSION BY REAL TIME PCR IN CANINE OSTEOSARCOMA CELL LINES FROM DOGS NORMAL AND INCREASED SERUM ALKALINE PHOSPATASE CONCENTRATION.** LCR Rodrigues[1], CM Piskun[1], V Thompson[1], TJ Stein[1]. [1]Department of Medical Science, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA

Osteosarcoma (OSA) arises from malignant osteoblasts in dogs and human and is highly metastatic and frequently develops chemotherapy resistance resulting in short survival time even for treated patients. An increase in serum alkaline phosphatase (ALP) concentration at diagnosis is a negative prognostic factor for OSA patients. We have generated canine primary OSA cell lines from OSA-bearing dogs with increased serum ALP concentration and have found these lines to be more resistant to chemotherapy in vitro than OSA cell lines from dogs with normal serum ALP. This study evaluated the expression of multidrug resistance genes in canine OSA cell lines generated from OSA-bearing dogs with normal and increased serum ALP concentration.

Six cell lines were used, 3 from OSA dogs with normal ALP, 3 from OSA dogs with increased ALP. Quantitative RT-PCR was used to measure the mRNA expression level of ABCB1, ABCC1, ABCG2, and ERCC1. The ribosomal 18S mRNA was used as an endogenous reference and RNA from normal bone was used to compare against.

OSA cell lines associated with increased serum ALP have an increased relative expression of ABCB1, ABCC1, ABCG2 and ERRC1. The most striking difference was a 17-fold increase in relative expression of ABCG2 between cell lines associated with increased and normal serum ALP (3.48 vs. 0.199, respectively). In addition, the difference in relative expression was 10-fold for ERCC1 (15.61 vs. 1.52) and 7-fold for ABCC1 (5.58 vs. 0.75). Finally, relative expression of ABCB1 was increased nearly two-fold (10.99 vs. 6.08) in cell lines associated with increased serum ALP compared to normal serum ALP cell lines. Despite these increases, the differences failed to meet statistical significance ($p < 0.05$) using a two-tailed Mann-Whitney test.

An increased serum ALP concentration is a negative prognostic factor in dogs with osteosarcoma. Similarly, cell lines from OSA-bearing dogs with increased serum ALP are more resistant to chemotherapy compared to cell lines from OSA-bearing dogs with normal serum ALP. We evaluated the relative expression level of multidrug resistance genes in OSA cell lines associated with different serum ALP concentration. We found increased relative expression of 4 multidrug resistance genes in OSA cell lines associated with increased serum ALP, though the differences failed to reach significance. One potential reason for the poorer prognosis in this population may be the differences in chemosensitivity, possibly due to differences in the expression of multidrug resistance genes. However, further studies are necessary to better understand this relationship.

**ABSTRACT O-10**

**CONTINUOUS LOW-DOSE (METRONOMIC) ORAL CHEMOTHERAPY OF CANINE APPENDICULAR OSTEOSARCOMA.** JP Woods[1], S Boston[1], AJ Mutsaers[1], DE Saam[1], B Coomber[2]. [1]Clinical Studies, [2]Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

Osteosarcoma is the most common primary bone tumour in dogs with most dogs dying of metastatic disease despite surgery and maximum tolerated dose (MTD) adjuvant chemotherapy. In veterinary oncology, continuous low-dose (metronomic) schedules of chemotherapy have become more popular for patient management. Therefore, the purpose of this study was to retrospectively investigate the effectiveness of the addition of low-dose metronomic chemotherapy following curative-intent amputation and adjuvant carboplatinum chemotherapy to dogs with appendicular osteosarcoma.

Dogs presented to the Ontario Veterinary College with a diagnosis of appendicular osteosarcoma who underwent limb amputation followed by adjuvant carboplatinum chemotherapy were eligible for this study. Dogs completed the amputation and carboplatinum and either received no further treatment (control); or continued with a metronomic chemotherapy protocol (treated) consisting of cyclophosphamide (10-25 mg/m$^2$ q 24-48h); nonsteroidal anti-inflammatory (NSAID); and doxycycline (5 mg/kg q24h).

Sixty-one dogs entered the study and 21 dogs were evaluable. The control group had 13 dogs with a median metastasis-free interval of 178 days and a median survival time of 504 days. The treatment group had 8 dogs with a median metastasis-free interval of 332 days ($P = 0.16$) and a median survival time of 340 days ($P = 0.55$).

Low-dose metronomic chemotherapy had few adverse effects; however, efficacy was difficult to determine perhaps due to lack of prospective randomization of the dogs into the groups. Future studies are needed to determine selection of metronomic agents (timing and dosing schedules); biomarkers for efficacy and toxicity; and combinations with standard drug dosing, newer targeted drugs, or immunotherapy.

**ABSTRACT O-11**

**SERUM C-REACTIVE PROTEIN CONCENTRATIONS IN CATS WITH ALIMENTARY LYMPHOMA UNDERGOING CHEMOTHERAPY.** SRR Lucas, VM Winkel, TLR Pavan. School of Veterinary Medicine and Animal Science – University of São Paulo, SP, Brazil

C-reactive protein (CRP) is an acute-phase protein that is used as a marker of infection and inflammation. Recently, it has been used as a predictor of survival in some neoplastic conditions in dogs and human beings, but there are few studies about CRP in cats. The goal of this study was to evaluate CRP during chemotherapy and its relation with remission in cats with alimentary lymphoma. CRP was measured by the use of a commercial species-specific immunoassay test (Elisa Kit Cat CRP- Icllab – USA)

in thirteen healthy cats and eight cats with alimentary lymphoma, confirmed by histopathologic examination. The eight cats with lymphoma did not receive any treatment prior the diagnosis. In healthy cats only one sample was collected. The cats with alimentary lymphoma were evaluated at 2-week intervals along 12 weeks, when they presented clinically stable. For both groups, samples were collected, harvested and frozen at -70°C until tested. Mann-Whitney test followed by multiple Tukey´s tests were used to compare the groups and weeks of treatment. Mean CRP concentration was significantly higher ($p = 0{,}0002$) in cats with alimentary lymphoma (270,8l±112,1µg/mL) at the diagnosis than in healthy cats (88,76±23,72µg/mL). At all other times while cats were in treatment, CRP concentrations decreased, specially at 12th week (181,9±86,63µg/mL) but the difference was not statistically significant ($p = 0{,}113$) in relation to the diagnosis. In conclusion, CRP concentration was higher in cats with alimentary lymphoma before chemotherapy and did not decrease to normal values during chemotherapy.

**ABSTRACT O-12**
**SERUM AMYLOID A CONCENTRATIONS IN CATS WITH LYMPHOMA DURING CHEMOTHERAPY.** VM Winkel, SRR Lucas, VABF Wirthl. School of Veterinary Medicine and Animal Science – University of São Paulo, São Paulo, SP, Brazil

Serum amyloid-A (SAA) is an acute-phase protein that has been used as a biomarker of inflammation, infection, neoplastic conditions and trauma, considered as a part of the innate host defense system. Lymphoma is one of the most common neoplasia in cats. The purpose of this study was to evaluate SAA concentrations at diagnosis and remission in cats with lymphoma. SAA was measured by the use of a commercial immunoassay kit (PHASETM Serum Amyloid A Assay (SAA) – Multispecies – Tridelta ltd - Ireland) in two groups of cats: 13 healthy cats and 16 cats with lymphoma (alimentary, mediastinal, renal and multicentric) without previous treatment, including the use of prednisone or chemotherapy. From the cats with lymphoma, 11 animals were evaluated during chemotherapy, at 2- week intervals along 12 weeks. For both groups, serum samples were collected and frozen at -70ºC until assayed. ANOVA test followed by multiple Tukey´s tests were used to compare the groups. The mean of SAA concentration was significantly higher ($p < 0{,}0001$) in cats with lymphoma (9,721±16,342µg/mL) at the diagnosis than in healthy cats (0,053±0,138µg/mL). SAA levels decreased when lymphoma remission was achieved, and was significantly different from 6th (0,798±0,895µg/mL) until 12th week (0,362±0,491µg/mL) of treatment ($p = 0{,}0076$), when compared to the moment of diagnosis. In conclusion, SAA concentrations decreased at remission in cats with lymphoma undergoing chemotherapy but the levels didn´t return to the normal values.

# SMALL ANIMAL – OTHER

**ABSTRACT OT-2**
**HOSPITAL VERSUS COMMUNITY ACQUIRED INTRA-ABDOMINAL INFECTION OF GASTROINTESTINAL ORIGIN IN DOGS.** ANK Floras, CR Sharp. Tufts Cummings School of Veterinary Medicine, North Grafton, MA

Intra-abdominal infections (IAI) are common and associated with high mortality rates in dogs. In human medicine, hospital acquired (HA)-IAI is more commonly associated with multidrug resistant (MDR) bacteria, and thus higher mortality, than community acquired (CA)-IAI. The purpose of this study was to determine whether significant differences in bacterial culture and susceptibility profiles and outcome exist between HA-IAI and CA-IAI in dogs. Medical records of dogs with IAI of gastrointestinal origin were retrospectively reviewed. Age, sex, breed, etiology of infection, culture & susceptibility, and outcome were recorded. Fifty three dogs were identified; 24 had CA-IAI, 8 had HA-IAI from another hospital, 13 developed HA-IAI in our hospital, 7 dogs had CA-IAI at the time of presentation and later developed HA-IAI, and 1 dog presented with HA-IAI and following source control developed another HA-IAI in our hospital. *E. coli* and *Enterococcus* spp. were isolated most frequently regardless of whether CA- or HA-IAI, however organisms associated with HA-IAI were more likely to be MDR. Twenty five dogs survived to discharge (47.2%), 20 (37.7%) were euthanized and 8 (15.1%) died. Dogs with HA-IAI that developed in our hospital were less likely to survive to discharge when compared with dogs presenting with CA-IAI, however this did not hold true for dogs that presented with HA-IAI from another hospital. Results suggest that dogs with HA-IAI are more likely to have infections with MDR bacteria and have a higher mortality than CA-IAI. The results of this study have implications for antibiotic choice and prognosis for dogs with HA-IAI.

**ABSTRACT OT-3**
**MULTI-CENTER EVALUATION OF THE ADMINISTRATION OF CROTALID ANTIVENOM IN CATS.** MB Pashmakova[1], MA Bishop[1], DM Black[1], SI Johnson[2], JW Barr[1]. [1]Texas A&M University, College Station, TX, [2]Emergency Animal Hospital of Northwest Austin, Austin, TX

Anecdotally cats are considered to be relatively resistant to snake envenomation; therefore, the collective experience with antivenom administration in cats is limited. The purpose of this study was to describe administration criteria, complication frequency, and overall survival following crotalid antivenom administration in cats.

Information about antivenom administration in cats from 2000 to 2011 was gathered from facilities that frequently manage crotalid envenomations. Medical records were searched for patient data including signalment, administration criteria, prophylactic medication, antivenom product, immediate reactions, and survival. A multiple-choice answer format was used to maximize response rate and facilitate data analysis. Preliminary data were analyzed and odds ratios were calculated using a Fishers exact test.

Sixty-eight of 203 (33.5%) envenomated cats received antivenom. The most frequently administered product was Antivenin (Crotalidae) Polyvalent (ACP) in 57/68 (83.8%) cats, followed by polyvalent F(ab')2 in 9/68 (13.2%) cats and Crotalidae polyvalent immune $F_{ab}$ (Crofab) in 2/68 (2.9%) cats. The most frequent indication was suspected rattlesnake envenomation in 66/68 (97.1%) cats, followed by hematologic abnormalities in 39/68 (57.4%) cats. Acute hypersensitivity reactions occurred in 15 cats (22.1%), of which 14 cats received ACP. Three cats died acutely, none of which received treatment with antihistamines or glucocorticoids before administration of antivenom (OR:19.4, 95% CI:1.0-395.1, $P = 0.0227$). Overall, 64/68 cats (94.1%) receiving antivenom survived to discharge.

In conclusion, antivenom was well tolerated in cats and survival following administration was excellent. Premedication may decrease frequency of reactions and improve survival; however, severity of illness rather than product administered may affect outcome.

**ABSTRACT OT-4**
**ENDOSCOPIC-GUIDED LASER ABLATION OF VESTIBULOVAGINAL DEFECTS IN 36 DOGS.** S Burdick, A Berent, C Langston, C Weisse. The Animal Medical Center, New York, NY

The purpose of this report is to describe and retrospectively evaluate short and long-term outcomes in female dogs after endoscopic-guided laser ablation (ELA) of vestibulovaginal septal defects.

36 female dogs with a persistent paramesonephric remnant (PPMR) ($n = 19$), vaginal septum ($n = 11$), or dual vagina ($n = 6$) were included. All patients had complete cystourethrography. Simultaneous diagnosis and treatment was performed via vaginoscopic guidance. ELA was used to transect the vaginal membrane. Patients with intramural ectopic ureters (EU) were concurrently treated with cystoscopic-guided laser ablation. A repeat endoscopy was performed in 18 dogs approximately 6 weeks post ELA and all vaginal and ureteral defects were re-assessed.

26 dogs presented for urinary incontinence, 2 for recurrent urinary tract infections (UTI) and 8 for both incontinence and UTI. Thirty dogs had concurrent EU. The diode laser (n = 28) or Holmium:YAG laser (n = 8) were used. Five dogs had mild postoperative dysuria, which resolved within 24 hours. One complication occurred (laser perforation of the vaginal wall). There were no negative effects from this event and at an 8 week re-scoping the tissue was fully healed. There was significant improvement in continence scores in all dogs ($p < 0.001$) and a decreased incidence of UTIs after laser correction of the congenital malformation(s) in all dogs ($p = 0.0287$). The median follow-up time was 33 months (2-49 months).

ELA provided an effective, safe and minimally invasive treatment for various vestibulovaginal septal remnants, avoiding the need for more invasive surgery for a disease that has an unknown significance.

## ABSTRACT OT-5

**$^{18}$FLT-PET/CT FOR NON-INVASIVE FUNCTIONAL IMAGING OF CANINE BONE MARROW.** JA Rowe[1], CJ LeBlanc[1], SA Kania[1], SJ Newman[1], M Akula[2], GD Galyon[1], MJ Long[2], SJ Kennel[2], GW Kabalka[2], AK LeBlanc[1,2]. University of Tennessee College of Veterinary Medicine;

[2]University of Tennessee Graduate School of Medicine, Molecular Imaging and Translational Research Program; Knoxville, TN

Non-invasive techniques to evaluate bone marrow function are needed to optimize care of dogs with suspected or confirmed hematopoietic disorders. Positron Emission Tomography/Computed Tomography (PET/CT) using $^{18}$F-fluoro-l-deoxythymidine ($^{18}$FLT), a PET tracer that physiologically concentrates in bone marrow and acts as a marker of cellular proliferation, offers a non-invasive imaging technique for whole-body assessment of marrow proliferation.

Three (3) clinically normal, purpose-bred dogs were imaged using $^{18}$FLT for assessment of bone marrow proliferation at baseline and at 7 and 14 days post administration of a single dose of cyclophosphamide (225 mg/m$^2$ PO) to nonselectively suppress hematopoiesis. PET/CT images were acquired with dogs under light general anesthesia following a minimum 12 hour fast. Regions of interest were drawn over sites of clinical relevance and increased tracer uptake, including common marrow sampling sites. Standardized uptake values were calculated utilizing dedicated analysis software. Bone marrow aspirates from both the proximal humerus and wing of the ilium were assessed within 24 hours of each imaging timepoint by cytology and flow cytometric cell cycle analysis for comparison with PET imaging findings. Cytologic analysis included estimation of overall cellularity with a 200-cell differential count discriminating cells as early erythroid, late erythroid, early granulocytic, late granulocytic, monocyte/macrophage, lymphocyte, plasma cell, or other. CBCs were assessed daily after cyclophosphamide administration.

Despite development of profound circulating neutropenia (median neutrophil nadir 580 cells/uL, 8 days post-cyclophosphamide administration), no significant changes in overall marrow cellularity were detected. Statistically significant changes between sampling timepoints were detected in the granulocytic index (ratio of early to late granulocytic precursors) in the wing of the ilium aspirates ($p = 0.007$), and flow cytometric cell cycle analysis was indicative of DNA insult at all sites sampled. Strong physiologic $^{18}$FLT uptake remained present in axial and proximal appendicular marrow sites. Changes over time in $SUV_{max}$ were significant ($p = 0.003$) in proximal humerus but not wing of ilium sites.

Reports in humans with myeloproliferative diseases demonstrate utility of $^{18}$FLT-PET in characterizing such diseases. Changes observed in bone marrow samples in this study preclude correlation between $^{18}$FLT signal and marrow function, likely due to cyclophosphamide-induced cell cycle arrest rather than cytotoxicity. Future studies using site-specific marrow ablative techniques are planned to produce more conspicuous marrow hypocellularity.

## ABSTRACT OT-6

**LIPOPOLYSACCHARIDE, TUMOR NECROSIS FACTOR, AND INTERLEUKIN-1B INDUCE NT-PRO C-TYPE NATRIUETIC PEPTIDE SECRETION FROM CANINE VASCULAR ENDOTHELIAL CELLS.** KA Osterbur, DH Yu, AE DeClue. University of Missouri College of Veterinary Medicine, Columbia, MO

The N-terminal portion of pro C-type natriuretic peptide (NT-pCNP) has shown promise as a biomarker for sepsis in humans and dogs. NT-pCNP is thought to be primarily secreted from the vascular endothelium, however the mechanism of NT-pCNP induction in dogs is unknown. We hypothesized that bacterial pathogen associated molecular pattern motifs (PAMPs) would induce NT-pCNP secretion from canine vascular endothelial cells to a significantly greater degree than inflammatory mediators. To test our hypothesis, we stimulated canine vascular endothelial cells with PAMPs (lipopolysaccharide, lipoteichoic acid, and peptidoglycan), inflammatory mediators (TNF, IL-1β, IL-6, CXCL-8, IL-10, IL-21, Interferon-γ, and Vascular Endothelial Growth Factor) or control (Phosphate Buffered Saline [PBS]). Statistics were performed using the Mann Whitney Rank Sum Test or Kruskal-Wallis One Way Analysis of Variance on Ranks with post-hoc Dunn's Method ($P < 0.05$ considered significant).

NT-pCNP concentrations were significantly greater after lipopolysaccharide (median, Q1, Q3; 11, 10, 11.5 pmol/L; $P = 0.024$), TNF (13.3, 12.1, 14.5 pmol/L; $P = 0.012$) and IL-1β (24.1, 19.4, 28.1 pmol/L; $< 0.001$) stimulation compared to PBS (1.7, 1.1, 2.1 pmol/L). The other stimulants did not result in NT-pCNP secretion compared to PBS. Lipopolysaccharide, TNF and IL-1β induction of NT-pCNP secretion were both dose and time dependent in nature.

Of the compounds tested, IL-1β, TNF, and lipopolysaccharide resulted in the greatest CNP secretion from canine vascular endothelial cells. This study provides insight into why dogs with sepsis have higher serum concentrations of NT-pCNP when compared to dogs with other forms of critical illness.

## ABSTRACT OT-7

**PENTOSAN POLYSULFATE AND N-ACETYL GLUCOSAMINE COMBINATION ENHANCES INHIBITION OF INTERLEUKIN-8 PRODUCTION BY MELOXICAM IN CANINE CHONDROCYTES.** KE Walker[1], AN Johnson[2], MW Grzanna[1], CG Frondoza[1,2,4]. [1]Nutramax Laboratories, Inc., Edgewood, MD, [2]Mississippi State University, Mississippi State, MS, [3]Johns Hopkins University, Baltimore, MD

The pathogenesis of osteoarthritis (OA) in humans and animals is characterized by excess production of pro-inflammatory mediators including cytokines and chemokines. Chemokines such as interleukin-8 (IL-8) are thought to play a role in OA by acting as signaling molecules and pain inducers. Meloxicam, a non-steroidal anti-inflammatory drug (NSAID), is commonly used to treat OA in dogs. However, its efficacy is hampered by life-threatening side effects raising the question whether dosing can be reduced through combining with other agents. Non-pharmacologic compounds, such as pentosan polysulfate (PPS) and N-acetyl glucosamine (NG), have been reported to promote chondrocyte anabolic activity and could be disease modifying. We hypothesized that the combination of [PPS + NG] enhances the effect of low concentrations of meloxicam on IL-8 production in canine chondrocytes.

Canine chondrocyte monolayer ($5x10^5$ cells/well) or microcarrier spinner cultures ($4x10^6$ cells/flask) were activated with interleukin-1 (IL-1 50ng/ml) for 24 hours and then incubated with: (a) control media alone, (b) a clinically relevant concentration of [PPS + NG] (AUPEN5000TM, PPS: 125μg/mL, NG: 200μg/mL), (c) a sub-therapeutic concentration of meloxicam (11.7ng/ml), or (d) a mixture of [PPS + NG] + meloxicam (11.7ng/ml) for an additional 24 hours. Cell culture supernatant was analyzed for IL-8 production by ELISA. Statistical analysis was run using one-way ANOVA and the Holm-Sidak method with significance set at $P < 0.05$.

Non-activated chondrocytes produced low levels of IL-8 (˜5000pg/ml) in both monolayer and microcarrier cultures. IL-1β activation increased IL-8 production (˜3-5 fold $P < 0.001$). The sub-therapeutic concentration of meloxicam decreased IL-8 production by ˜20-30% ($P < 0.001$). Treatment with [PPS + NG] reduced cytokine-induced IL-8 production by ˜20-50% ($P < 0.001$.) The combination of [PPS + NG] with meloxicam showed a more profound decrease in IL-8 production by ˜50-70% ($P < 0.001$) in both monolayer and microcarrier cultures.

Here we report that [PPS + NG], when combined with a sub-therapeutic concentration of meloxicam, potentiates the inhibition of IL-8 production in canine chondrocytes. Our finding suggests that this combination strategy may minimize adverse side effects while maintaining anti-inflammatory activity (Research supported by Nutramax Laboratories, Inc.).

## ABSTRACT OT-8

**COMPARISON OF PCR- AND CULTURE-BASED METHODS TO EVALUATE BLOOD FROM DOGS WITH SUSPECTED SEPSIS.** RM Heilmann[1], PG Xenoulis[1], J Barr[2], SE Dowd[3], JS Suchodolski[1], JM Steiner[1]. [1]Gastrointestinal Laboratory and, [2]Small Animal Veterinary Teaching Hospital, Texas A&M University, College Station, TX, [3]Molecular Research DNA Lab, Shallowater, TX

Sepsis is associated with a poor outcome in critically ill dogs. Conventional blood culture, the current gold standard method for the detection of bacteremia in veterinary medicine, is associated with a long turnaround time, a low sensitivity for some organisms, and often fails to grow bacteria after initiation of antimicrobial treatment. Polymerase chain reaction (PCR)-based diagnostics may have a high sensitivity for detecting bacterial DNA in the bloodstream allowing early initiation of appropriate antimicrobial therapy, which may improve patient outcome. Molecular techniques have been considered as ancillary tools for the detection of bacteremia and identification of pathogens in humans and also in dogs with bacterial endocarditis, but have not been reported in dogs with suspected sepsis. The aim of this study was to compare PCR analysis and culture of blood for the detection of bacteremia in dogs with suspected sepsis.

Six dogs with suspected sepsis (median age [range]: 4 [1-10] years; 4 males, 2 females), referred to the Emergency Service at Texas A&M University, and 6 healthy control dogs (median age [range]: 4 [0.8-10] years; 2 males, 4 females) were prospectively enrolled. Sepsis was suspected in dogs that had $\geq 2$ of the established systemic inflammatory response syndrome [SIRS] criteria: hypo- or hyperthermia, tachycardia, tachypnea, leukopenia or leukocytosis, and/or an increased number of band neutrophils. Patients included in the study were diagnosed with primary gastrointestinal ($n = 2$), systemic ($n = 2$), respiratory ($n = 1$), or pleural ($n = 1$) disease; four (66.7%) of the 6 dogs with suspected sepsis had received an antimicrobial drug within 1 week prior to sample collection. Two serial samples were obtained from 1 patient. Venous blood was collected aseptically for anaerobic and aerobic blood culture. Also, an aliquot was placed into an EDTA-tube, centrifuged, and blood cells were separated from plasma. DNA was extracted using a commercial kit and included a negative extraction control. Extracted blood cells and cell-free plasma were analyzed separately by PCR using universal 16S rRNA gene primers followed by 454-pyrosequencing of the PCR products.

One (14%) of the 7 blood cultures from patients with suspected sepsis was positive for *Enterococcus* spp. and an unidentified gram-negative bacterial strain, whereas none of the healthy controls had a positive blood culture. PCR was negative for all extracts from blood cells and cell-free plasma collected from patients with suspected sepsis and from healthy controls.

This study does not support that PCR-based techniques used directly on blood could serve as an alternative to conventional blood culture for the diagnosis of bacteremia in patients with suspected sepsis. However, further studies with larger numbers of dogs are needed to confirm these findings.

## ABSTRACT OT-9

**METAGENOMIC INVESTIGATION OF THE ORAL MICROBIOME OF HEALTHY DOGS.** JW Stull, A Sturgeon, M Costa, AS Peregrine, J Sargeant, JS Weese. Ontario Veterinary College, Guelph, Ontario, Canada

The body harbors complex and diverse bacterial populations (microbiomes) that are important for health and disease but which have been minimally investigated. Of particular interest is the oral microbiome, as it has been implicated in several diseases of varying anatomical locations including dental, respiratory, and cardiovascular disease, as well as cancer. The development of next generation sequencing platforms and advanced bioinformatics tools has allowed for proper characterization of these vast microbial populations. The objectives of this study were to determine the bacterial flora and diversity of the canine oral cavity.

Six dogs from different households were conveniently selected and an oral sample was taken by brushing the inside mouth with a sterile toothbrush. DNA was extracted and purified, 16S rRNA gene PCR performed and PCR products sequenced using next generation sequencing.

Over 10,000 sequences with mean lengths > 500 base pairs were amplified from each sample. Relative abundance of bacterial phyla in samples were similar among the dogs, with Bacteroidetes accounting for over 70% of each dogs' oral flora, followed by Proteobacteria (4-19%), Firmicutes (2-10%), and Fusobacteria (0.5-9%). Unclassified phylum-level sequences were uncommon (< 0.02%). Dogs varied in the number of genera identified (range 39-81). *Porphyromonas* and *Capnocytophaga* were identified in all dogs and were the most abundant genera in most dogs (45-84% and 5-46%, respectively). Eleven different *Porphyromonas* species were identified from the dogs. Nine zoonotic species were identified with $\geq$ 3 species present in each dog; *Capnocytophaga canimorsus* and *C. cynodegmi* were the most common. *Streptobacillus moniliformis* (causative agent of rat-bite fever) was identified in one dog.

Our methods amplified a large number of long sequences with high organism identity, revealing the utility of this technique for investigating the canine oral flora. The high genera diversity identified far surpassed culture-based studies, demonstrating a diverse and complex oral microflora. All dogs carried multiple zoonotic bacterial species, highlighting the zoonotic potential of the oral flora of healthy dogs. These results are in contrast with culture-based studies and indicate both a greater degree of complexity than has been previously identified and the abundance of organisms often reported as absent or rare. These data provide insight into the oral microbiome of healthy dogs; a critical step in understanding the role of oral flora in disease.

## ABSTRACT OT-10

**EFFECTS OF SPECIMEN TYPE (SERUM OR PLASMA) ON BIOCHEMISTRY RESULTS IN HEALTHY CATS.** BS Reynolds, C Brosse, M Legras, HP Lefebvre. Clinical Research Unit, National Veterinary School, Toulouse, France

Consecutive blood collection using different tubes can be difficult in cats. Serum or heparinized plasma is usually recommended for biochemistry but the potential effect of other anticoagulants is poorly documented in cats. The objective of this study was therefore to compare the concentration of routine chemical variables in serum or plasma obtained using various anticoagulants.

Eleven healthy cats were used. Blood was drawn from a jugular vein into evacuated tubes without or with anticoagulants (Li-heparin, EDTA and citrate). After centrifugation, serum/plasma was assayed by use of dry slide technology analyzer for glucose, urea, creatinine, sodium, potassium, chloride, total $CO_2$, calcium, phosphate, proteins, albumine, ALT and ALP. Effects of specimen type on results were assessed by analysis of variance.

Results obtained from serum and heparinized plasma were statistically different for 4/13 variables, but the observed differences were clinically irrelevant. For EDTA plasma, potassium and calcium could not be assayed. Other results were not statistically different or very close from those obtained from heparinized plasma or serum. Results obtained from citrated plasma were strongly divergent except for albumin, ALT and ALP.

In conclusion, EDTA plasma could be an appropriate alternative if needed for most biochemistry assays in cats.

**ABSTRACT OT-11**
**COMPULSIVE CAT ADOPTERS: EVALUATION OF OWNERS´ MENTAL HEALTH STATUS AND ATTACHMENT TO PETS IN A POPULATION OF BRAZILIAN OWNERS OF MULTIPLE CATS.** D Ramos[1], A Reche-Junior[1], NO Cruz[1], JAE Hernandez[2]. [1]Department of Medical Clinics, Faculty of Veterinary Medicine and Animal Science, University of São Paulo, Brazil, [2]Department of Fundamentals of Psychology, Institute of Psychology, State University of Rio de Janeiro, Brazil

The acquisition of domestic cats as pets constitutes a worldwide phenomenon. In highly populated areas such as São Paulo region of Brazil, keeping a less demanding pet such as a cat better suits the busy daily routines people tend to have. For some individuals, however, satisfaction seem to come from keeping many cats at once, a condition frequently considered to be a matter of animal hoarding, deleterious for both parts, the cats and the owner. In order to explore the mental health status of such individuals, a survey was conducted with 30 owners of multiple cats (i.e. 20 cats or more – group 1). Comparative groups 2 and 3 were composed by 30 owners of 1-2 cats and 30 participants who did not own any pet, respectively. A questionnaire composed by the SI-R (Saving Inventory-Revised), HAD (The Hospital Anxiety and Depression Scale) and the LAPS (Lexington Attachment to Pets Scale), all in Portuguese language, were given to the participants to answer. Results indicated more cases of anxiety among the non pet owners (i.e. 9 in 30 – versus 4 in 30 in both groups 1 and 2) and more depression cases among the owners of multiple-cats (i.e. 4 in 30 – versus 1 in 30 in group 1and 2 in 30 in group 3). Owners of multiple cats showed higher levels of attachment to pets, but their SI-R final scores did not differ from the other groups. In several ways, owning many pets seems to impair the social aspect of the owner´s life which can lead to isolation – this maybe the reason why more depression was seen in group 1. At least for the owners of this study, acquiring many cats maybe analogous to the habit of collecting "things", but yet at a non-pathological degree as from the questionnaire there was no evidence of animal hoarding. Alternatively, animal hoarding does not necessarily imply hoarding in general (as evaluated in the SI-R).

# SMALL ANIMAL - PHARMACOLOGY

**ABSTRACT P-1**
**THE PHARMACOKINETICS OF SINGLE DOSE EXTENDED RELEASE KEPPRA® WITH AND WITHOUT FOOD IN HEALTHY ADULT DOGS.** MJ Beasley[1], DM Boothe[2]. [1]Mississippi State University College of Veterinary Medicine, Mississippi State, MS, [2]Auburn University College of Veterinary Medicine, Auburn, AL

Levetiracetam appears to be a safe and potentially effective anticonvulsant for the control of epilepsy in dogs. However, its efficacy is challenged by a short half-life (often less than 2 hours). This half-life often results in subtherapeutic concentrations during an 8-hour dosing interval despite high oral doses. An extended release oral preparation recently has been approved in humans, which allows less frequent dosing intervals. The purpose of this study was to evaluate the pharmacokinetics of extended release levetiracetam (Keppra XR®) tablets in normal healthy dogs after a single oral dose with the goal of establishing a dosing regimen for the commercially available tablet that would allow 12 to 24 hour dosing intervals. The effect of food on drug movement was also evaluated.

Twelve client-owned dogs weighing at least 15 kg were studied using a crossover design. All dogs received intravenous levetiracetam, followed by a 24-hour washout period and then extended release levetiracetam orally (6 each with food or following a 12 hour fast; selection was random). All doses averaged 32.67 mg/kg. Blood samples were collected for 24 or 36 hours after intravenous or oral administration, respectively. Serum levetiracetam was quantitated using an ARK Diagnostic Levetiracetam Assay®. Data was subjected to non-compartmental pharmacokinetic analysis.

Keppra XR® was well tolerated. Serum levetiracetam reached the targeted therapeutic range (5 ng/mL) within 100 or 200 minutes and stayed within the range for an average of 19.8 hours (range 15-24.2) or 20.7 hours (range 16.7-28.7), in fasted and fed animals, respectively. Bioavailability and time to maximum concentration was significantly higher in fed versus fasted dogs, however, half-life was not significantly different between groups. This study supports 12 hour dosing intervals for levetiracetam when administered as Keppra XR® at approximately 30 mg/kg in dogs; 24 hour intervals may be acceptable for some dogs, although monitoring is recommended to confirm.

**ABSTRACT P-2**
**LOCAL AND SYSTEMIC EFFECTS OF A COMMERICAL PREPARATION OF CHONDROTIN SULFATE, HYALURONIC ACID AND N-ACETYL-D-GLUCOSAMINE WHEN ADMINISTERED INTRA-ARTICULARLY OR INTRAMUSCULARLY TO HEALTHY DOGS.** CM MacPhail, MM Brewer, JR Hawley, JK Veir, MR Lappin. Department of Clinical Sciences, Colorado State University, Fort Collins, Colorado

Products containing glycosaminoglycans (GAG) are frequently administered by various routes to dogs to aid in the management of osteoarthritis (OA). However, little information is available concerning effects of these products when administered to dogs, particularly regarding biomarkers of OA. The purpose of this study was to determine local and systemic effects of a single intra-articular (IA) administration or repeated intramuscular (IM) administrations of a formulation of chondroitin sulfate, hyaluronic acid, and N-acetyl-D glucosamine in healthy dogs.

Eight healthy, young adult, mixed sex beagles were used in this study. Using procedures approved by the Institutional Animal Care and Use Committee, blood, serum, and synovial fluid (both stifle joints) were collected on Days -7, 0, 3, 7, 14, 35, 42, 49, 56, and 63. After arthrocentesis on Day 0, 1.5 ml of Polyglycan® (Arthrodynamic Technologies, Lexington, KY) was injected into the right stifle joint. Days 14 to 35 were designed as a washout period. After sample collection on Day 35, Polyglycan® was administered IM (0.1 ml/lb) to each beagle with repeated IM injections on Days 42, 49, and 56. Complete blood cell counts (CBC) and serum biochemical profiles were performed on Days -7, 0, 35 and 63. Aggrecan synthesis (epitope CS846), GAG, and prostaglandin E2 (PGE2) concentrations from serum and the synovial fluid of the treated stifle collected at all time points were determined using previously validated assays. The dogs were observed daily for changes in appetite, attitude, ambulation, and body temperature.

Other than evidence of transient lameness (< 24 hours) following synovial fluid sampling, no other clinical side-effects were noted. There were no significant changes in CBC or biochemical results over time. There were no significant changes in serum or synovial fluid concentrations of CS846 or GAG over time. While there were no significant changes in serum concentrations of PGE2 over time, synovial fluid concentrations of PGE2 were significantly lower on Day 56 ($p = 0.03$) and Day 63 ($p = 0.02$) when compared to Day 35 concentrations.

The results indicate administration of Polyglycan® using these routes of administration and dose schedule had no measurable side-effects. Repeated arthrocentesis in the presence of these Polyglycan® protocols had no measurable effect on serum concentrations of aggrecan synthesis, GAG, and or PGE2 or synovial fluid concentrations of aggrecan synthesis or GAG. However repeated IM injection resulted in a significant decrease in synovial fluid PGE2 concentrations, which may reflect a positive influence on synovial inflammation.

**ABSTRACT P-3**
**EFFECTS OF ORAL CYCLOSPORINE ON CANINE T-CELL EXPRESSION OF IL-2 AND IFN-γ ACROSS A 12-HOUR DOSING INTERVAL.** C Fellman, T Archer, J Stokes, K Lunsford, A Mackin. Mississippi State University College of Veterinary Medicine, Starkville, MS

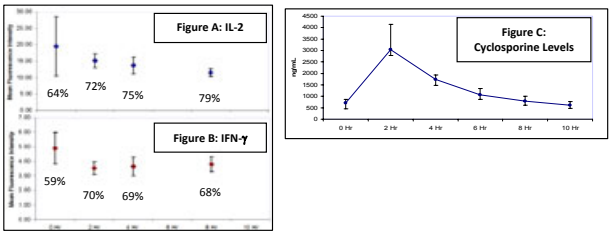
Cyclosporine is a commonly used immunosuppressive drug in dogs, but dosing is often empirical and based on clinical

response. Pharmacokinetic monitoring of blood drug levels is performed, but often does not reliably predict clinical response. Pharmacodynamic assays measuring the effects of the drug on target cells more reliably predict immunosuppressive effectiveness in humans. Our laboratory has developed pharmacodynamic assays for measuring the effects of cyclosporine on canine T-cell cytokine production. The current study evaluates the effects of cyclosporine on IL-2 and IFN-γ expression across a 12-hour dosing interval using flow cytometry, and compares these values to traditionally-measured drug levels at similar time points.

Oral cyclosporine was given to 6 healthy dogs at 10 mg/kg every 12 hrs for 8 days. Blood was drawn before treatment (baseline), and then on day 8 at 0, 2, 4, 6, 8, and 10 hrs. Cyclosporine levels were measured post-treatment via immunoassay. For cytokine assays, blood was incubated with PMA and ionomycin, CD3 was used to label T-cells, and intracellular staining of IL-2 and IFN-γ was performed. Samples were evaluated with a FACSCalibur flow cytometer, and the mean fluorescence intensity of 5,000 lymphocytes was measured at baseline and day 8 (0, 2, 4 & 8 hrs).

Flow cytometry reflected consistent suppression of IL-2 and IFN-γ expression at all time points across the dosing interval (Figures A&B). At trough drug levels, cytokine expression was most variable, but was still markedly decreased from pre-treatment levels. Blood cyclosporine levels were highly variable among dogs, but still displayed a characteristic peak and trough (Figure C).

Suppression of IL-2 and IFN-γ was consistent among all dogs tested, and the slightly more variable cytokine suppression at 0 hrs was reflective of decreased trough drug levels. Further testing of pharmacodynamic cytokine assays to assess their utility in clinic patients is indicated.




Figures presented as mean +/- standard deviation.IL-2 Baseline MFI 54.2; IFN-c Baseline MFI 11.9.

Percentages superimposed below data points on Figures A&B represent percentage reduction of mean MFI from these original baseline values.

# SMALL ANIMAL – RESPIRATORY

**ABSTRACT R-1**

**COMPARISON OF BRONCHOALVEOLAR LAVAGE ASPIRATION TECHNIQUES IN HEALTHY DOGS: MANUAL ASPIRATION AND SUCTION PUMP ASPIRATION.** KS Woods, AMN Defarges, ACG Abrams-Ogg, H Dobson, L Viel, D Bienzle. Ontario Veterinary College University of Guelph, Guelph, ON, Canada

The purpose of the study was to compare the diagnostic quality of bronchoalveolar lavage fluid (BALF) cellular component acquired by manual aspiration via polyethylene tubing (MAPT) and suction pump aspiration (SPA) via suction trap connection techniques in healthy dogs.

Bronchoalveolar lavage was performed under general anesthesia using bronchoscopic guidance in twelve healthy beagles. Following passage of the endoscope, sterile polyethylene tubing was inserted through the biopsy channel and MAPT performed with a 35mL syringe attached to the tubing wedged in the bronchus. Similarly, SPA was performed using 5kPa negative pressure applied directly to the bronchoscope's suction valve via disposable aspiration tube. MAPT and SPA techniques were performed in randomized order in opposite caudal lung lobes of each dog. Two lavages boluses were infused at each site using a weight-adjusted aliquot volume (1 mL/kg). The BALF samples were submitted and prepared for total nucleated cell count and differential cell count. A subjective scale for quality and percent of retrieved fluid were evaluated. Results were compared using a paired Wilcoxon sign rank test.

The proportion of BALF retrieved was significantly higher (mean difference 19.02%, $p = 0.001$) for SPA than MAPT. From the BAL cell differential analysis, a higher percentage of epithelial cells was present when compared to SPA (mean difference = 0.81, $p = 0.05$).

The results suggest that SPA provided BALF samples of higher diagnostic quality than MAPT in healthy dogs. The SPA technique may improve the diagnostic accuracy and significance of BAL in dogs.

Statement on prior publication: Abstract presented at the Ontario Veterinary College Graduate Research Symposium, University of Guelph, Guelph, Ontario, Canada; November 16th, 2011.

**ABSTRACT R-2**

**ENVIRONMENTAL AIR QUALITY, METEOROLOGICAL FACTORS, AND FELINE RESPIRATORY DISEASE.** RH Stone[1], M Mason[2], JR Smith[1]. [1]College of Veterinary Medicine and, [2]College of Public Health, University of Georgia, Athens, GA

Poor air quality and temperature extremes increase overall human mortality and hospitalization; the effect on animal health is undetermined. This study investigated if feline respiratory disease is associated with air quality or meteorological parameters.

Retrospective case review for adult, coughing cats. Monthly meteorological and Environmental Protection Agency data were gathered for 14 cities. Relationships between environmental variables were assessed with Pearson correlation coefficients. Association between environmental variables and respiratory visits was evaluated with Poisson regression analysis. Demographic data were compared using ANOVA and Chi square analysis.

Of 594,032 primary care visits, 10,483 involved coughing. Respiratory diagnoses included asthma (1,352); bronchitis (853); tracheitis (481); laryngitis (46) and pneumonia (12). Siamese cats were overrepresented in coughing and asthma/bronchitis groups (RR 1.6 [CI 1.3-1.8], $p < 0.0001$). A negative association existed between ambient temperature and coughing (6.0% increase in visits/ 10°F temperature drop, $p < 0.0001$). A positive association was demonstrated for relative humidity and coughing (7.0% increase in visits/ 10% rise in humidity, $p < 0.0001$). For cats with asthma or bronchitis, only a negative association between ambient temperature and cough was found (9.0 % increase in visits/10°F temperature drop, $p < 0.0001$). No significant association was found for particulate or ozone concentrations. The city of residence was significant in all models ($p < 0.0001$).

In conclusion, relative frequencies of visits for coughing, asthma or bronchitis were associated with seasonal variables. The significant variation in respiratory caseload between cities demonstrates that other seasonal and regional air quality factors may impact feline respiratory health.

**ABSTRACT R-3**

**TRACHEAL MALFORMATIONS IN YORKSHIRE TERRIERS.** E Branter, C Weisse, A Berent. Animal Medical Center, NY, NY

Tracheal collapse is a syndrome describing various forms of functionally narrowed tracheal lumens. Focal tracheal cartilage malformations in Yorkshire Terriers is a previously unclassified manifestation of this syndrome.

The purpose of this study was to describe the clinical signs, diagnostic findings, and short-term outcome following endoluminal tracheal stenting for this unique population. Medical records were reviewed of all dogs presenting to the Animal Medical Center Interventional Radiology Service with a diagnosis of tracheal malformation. Clinical signs including dyspnea, honking/raspy breathing, and coughing were each serially classified by the owner on a scale of 0-10 (absent to continuous). Outcomes and complications were reported.

Thirteen dogs, all Yorkshire Terriers, were identified to have non-traditional cartilage malformations of the trachea. Thoracic radiographs found focal collapse at the thoracic inlet in all dogs. All dogs had tracheal stent placement. Tracheoscopy was per-

formed in 11/13 dogs and all of these had evidence of "W" shaped tracheal rings. Median pre-stent coughing score was 2 (0-10), post-stent was 2 (0-2), median pre-stent honking score was 6.5 (0-10) and post-stent was 0 in all, median pre-stent dyspnea was 5 (2-10) and post-stent was 0 (0-2). Complications occurred in 5/13; 2 stent fractures not requiring intervention and 3 tissue ingrowth (1 resolved with prednisone, 2 required stent placement).

A subset of Yorkshire Terriers exists with a unique form of the tracheal collapse syndrome that can be identified pre-operatively and is treated in similar ways with expected outcomes and complications similar to those reported with other forms tracheal collapse.

**ABSTRACT R-4**
**ORAL GLUCOCORTICOIDS MAY DIMINISH EFFICACY OF ALLERGEN-SPECIFIC IMMUNOTHERAPY IN EXPERIMENTAL FELINE ASTHMA.** CH Chang, AE DeClue, LA Cohn, H Liu, CR Reinero. University of Missouri College of Veterinary Medicine, Columbia, MO

Allergen-specific rush immunotherapy (RIT) shows promise to treat asthma; however, pet cats would likely require concurrent glucocorticoids (GC) to control serious clinical signs. How immunosuppressive effects of GC would impact RIT is unknown. We hypothesized that oral but not inhaled GC will diminish efficacy of RIT in experimental feline asthma.

Cats (n = 6/group) were sensitized using Bermuda grass allergen (BGA) and randomized to receive BGA-specific RIT for 9 months with oral GC (prednisolone 10 mg SID), inhaled GC (budesonide 600ug SID) or placebo for the first 6 months. Bronchoalveolar lavage fluid (BALF) % eosinophils and other immunologic assays were performed.

Eosinophilic airway inflammation was suppressed similarly in all groups at month 6 (oral GC 5±2%; inhaled GC 13±4%; placebo 7±2%; p = 0.176). At month 9, % BALF eosinophils did not differ significantly between groups (oral GC 25±8%; inhaled GC 13±4%; placebo 11±5%; p = 0.084). However, 2 cats receiving oral GC/RIT at month 9 had markedly increased % BALF eosinophils (46% and 54%); comparatively, the highest % BALF eosinophils in the inhaled GC group was 23% and in the placebo/RIT group was 29%. No significant differences were found between groups at month 6 or 9 for lymphocyte blastogenesis, % blood Tregs, numbers of IL-10 producing cells or serum BGA-specific IgE.

While there was no statistically significant effect of oral or inhaled GC concurrent with RIT on airway eosinophilia, a subpopulation of cats treated with oral GC had diminished efficacy of RIT. Thus, inhaled GC may be a better option for asthmatic cats receiving RIT.

**ABSTRACT R-5**
**FELINE T REGULATORY CELLS IN BRONCHOALVEOLAR LAVAGE FLUID AND WHOLE BLOOD IN HEALTHY AND EXPERIMENTALLY ASTHMATIC CATS.** CH Chang, BD Eason, AE DeClue, CR Reinero. University of Missouri College of Veterinary Medicine, Columbia, MO

Regulatory T cells (Tregs) dampen harmful immune responses in allergic disease and are postulated to be deficient in number and/or function in allergic asthma. Evaluation of local Tregs in the feline respiratory tract has not previously been described. The purpose of this study was to develop a flow cytometric assay to measure Tregs in bronchoalveolar lavage fluid (BALF) and whole blood (WB), and to compare the %Tregs between healthy control (n = 6) and experimentally asthmatic cats (n = 8).

Asthma was experimentally induced using Bermuda grass allergen and samples collected 24 h after allergen aerosol challenge. Cells were stained with feline-specific and feline cross-reactive antibodies against CD4, CD5, and foxP3. A CyAn flow cytometer was used. The gating strategy involved identifying CD5+ cells on a CD5 vs SSC plot; these cells were analyzed for double staining with CD4 and foxP3 in a dot plot. The %Tregs was compared between groups and between WB and BALF within groups using a Mann-Whitney Rank Sum Test with $P < 0.05$ considered significant.

Results (median, range) showed detectable CD5+(CD4+foxP3+) Tregs in WB (controls: 4.1%, 1.9-4.9%; asthmatics: 3.3%, 1.9-5.8%) and BALF (controls: 4.1%, 1.6-8.9%; asthmatics: 6.7%, 3.3-9.2%) with no significant differences between groups (P = 0.943). The %Tregs was significantly higher in BALF compared with WB in asthmatic (P = 0.015) but not healthy (P = 0.931) cats.

In conclusion, flow cytometry is useful to identify feline Tregs. Experimentally asthmatic cats have significantly more local than peripheral Tregs, perhaps suggesting attempts to increase this regulatory population to dampen aberrant airway inflammation.

**ABSTRACT R-6**
**LUNG PATHOLOGY ASSOCIATED WITH TOXOCARA CATI INFECTION IN CATS IS INDEPENDENT OF DEVELOPMENT OF ADULT INTESTIONAL PARASITES.** AR Dillon, DM Tillson, J Hathcock, B Brawner, R Cattley, A Wooldridge, B Welles, S Barney, L Botzman, M Sermersheim. Auburn University, College of Veterinary Medicine, Auburn, AL

Lung disease associated with migrating T cati larvae in the cat has not been defined as to the timing of the arrival of the larvae in the lung, the nature of induced lung disease, or the correlation of lung disease to relevant diagnostic techniques. The purpose of this study was to describe the progression of T cati-induced lung lesions in kittens and adult cats, and determine if medication prescribed to prevent intestinal roundworm development alters the early larval migration through the lung.

Cats in infected groups were administered 5 oral doses of L3 T cati larvae. Four month old Specific Pathogen Free (SPF) kittens were randomly divided into three groups (6/group): an infected untreated group, an uninfected untreated control group, and an infected treated group (topical moxidectin and imidacloprid at the label dose, Advantage Multi for Cats, Bayer Animal Health). Six 2-3 year old adult multiparous female SPF cats were used as an infected untreated adult group. Cats were evaluated for the progressivity of the lung disease by serial CBCs, BALs, fecal examinations, thoracic radiographs, and thoracic CTs. Cats were euthanized 65 days after the initial infection and samples of intestine, liver, and fixed perfused lung were obtained. Lung samples were collected for bronchial ring reactivity via in vitro challenge. Adult worms in the intestine were collected for determination of sex and length.

Adult T. cati, of all stages, were recovered in infected untreated kittens (5/6) and infected untreated adults (5/6) in numbers consistent with spontaneous natural infections; most but not all cats with adult worms had eggs identified in the feces. No adult worms were identified in the uninfected controls or the infected treated group. All cats in the infected groups, including treated cats and untreated cats which did not develop adult worms, had lung pathology based on histopathology, radiographs, and CT evaluation.

Evaluation of the serial data in the infected cats demonstrated a peripheral eosinophilia (CBC) and eosinophilic cytology (BAL), which usually preceded radiographically identifiable lung lesions. Lung lesions were identified by CT on day 10 after infection and were progressive in sequential CT evaluations. Disease was not uniformly distributed among lobes or within lung lobes. Thoracic radiographs showed a classic diffuse bronchial-interstitial pattern. Pulmonary arterial, bronchial and interstitial disease were prominent histological lesions. Based on statistical evaluation of the histopathologic and CT lung scores, infected treated cats had a subtle attenuation but not prevention of lung disease compared to infected cats. Significant lung disease in kittens and adult cats is associated with the early arrival of T cati larvae in the lungs and is independent of the development of adult worms in the intestine.

**ABSTRACT R-7**

**CERVICAL LUNG LOBE HERNIATION IN DOGS WITH COUGH AS IDENTIFIED BY FLUOROSCOPY.** LA Nafe, ID Robertson, EC Hawkins. North Carolina State University College of Veterinary Medicine, Raleigh, NC

Dogs with cervical lung lobe herniation (CLLH) have been described in few case reports. In our experience, CCLH is common in dogs with cough. The purpose of this study was to characterize CLLH in dogs evaluated at a referral hospital. We hypothesized that CLLH would be a frequent finding in coughing dogs, and that herniation would be associated with a chronic cough and with intra-thoracic large airway collapse. Study dogs underwent fluoroscopic evaluation of cough from 2008-2010. CLLH was diagnosed by the transient displacement of lung cranial to a line between the manubrium and the first thoracic vertebra. Cases were eliminated if cough or forced expiration did not occur during fluoroscopy. Patient discharge summaries and reports of thoracic radiographs and fluoroscopy were reviewed retrospectively. Findings were compared between dogs with CLLH and without CLLH (controls) using routine statistical methods, $P < 0.05$ considered significant. Of 121 study dogs, 85 (70%) had CLLH. There was no significant difference in median duration of cough between dogs with and without CLLH (12 months and 10 months, respectively; $P = 0.792$). Duration of cough was < 1 month in 8 (9%) dogs with CLLH. No associations were found between signalment, body weight, presence of heart murmur, or the radiographic findings of cardiomegaly or left atrial enlargement, and CLLH. Collapse of the intra-thoracic trachea, mainstem bronchi, or either during cough as identified fluoroscopically were associated with CLLH ($P = 0.001$, 0.004, and 0.041, respectively). The extra-thoracic trachea was noted to kink during herniation in 33 (39%) of dogs with CLLH. CLLH was present in the majority of cases evaluated fluoroscopically for cough and was associated with intra-thoracic large airway collapse, but not duration of cough. Kinking of the trachea during herniation may contribute to progression of cough in some patients.

**ABSTRACT R-8**

**ASSESSMENT OF ARTERIALIZATION OF PERIPHERAL VENOUS BLOOD IN WELL-PERFUSED DOGS.** Y Shiroshita[1,2], Y Yamane[1]. [1]Department of Veterinary Surgery, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan, [2]Sagamigaoka Animal Clinic, Kanagawa, Japan

Multiple arterial blood gas samples are essential for monitoring a patient during perioperative and critical care. In small animals, an indwelling arterial cannula is usually used for multiple blood gas sampling, although it may cause massive hematoma from rupture of the artery. In well-perfused humans, blood obtained from a superficial hand vein after heating the hands in hot water for several minutes has been reported as an appropriate substitute for arterial samples for the measurement of pH, $PCO_2$, and lactate. The heated-hand technique is referred to as arterialization. Multiple blood gas samples can be obtained more easily and safely with this technique than with an indwelling arterial cannula. This study aimed to ascertain if arterialized venous blood samples can replace arterial blood gas sampling in well-perfused dogs.

Blood samples from the femoral artery (A), cephalic vein (CV), and saphenous vein (SV) were simultaneously collected from 8 lightly anesthetized dogs under conditions of experimentally induced metabolic acidosis, metabolic alkalosis, respiratory acidosis, and respiratory alkalosis in each dog. Samples were analyzed for pH, $PCO_2$, $PO_2$, [$HCO_3-$], and base excess for each acid–base status. Metabolic acidosis was induced by continuous infusion of 0.6 M $NH_4Cl$ in 5% dextrose, and metabolic alkalosis was induced by administration of 1 M sodium bicarbonate solution. Respiratory acid–base disturbances were induced by mechanical ventilation. The forepaw and hindpaw were continuously heated at 37°C to arterialize CV and SV. Mean arterial pressure was maintained at more than 80 mm Hg. Statistical analysis was performed using MANOVA and linear regression. Bland–Altman plots were used to assess the degree of agreement between the blood gas values of A and arterialized CV or SV. $P < 0.05$ was considered statistically significant.

pH, [$HCO_3-$], and base excess values in arterialized CV and SV exhibited no significant differences from those in A, high correlations with those in A (coefficient of determination, $r^2 = 0.93$–0.99), and a clinically approximate agreement with those in A, respectively. $PO_2$ values under all acid-base statuses in arterialized CV and SV were significantly lower than those in A ($P < 0.01$) and not correlated with those in A ($r^2 = 0.51$–0.75), respectively. $PCO_2$ values under metabolic acidosis in arterialized CV and SV were significantly higher than those in A [CV, 38.7 ± 2.6; SV, 37.5 ± 2.6; A, 33.2 ± 3.0 (mean ± SD) mm Hg; $P < 0.01$]. However, $PCO_2$ in arterialized SV was closely correlated with that in A under both metabolic and respiratory acid–base disturbances ($r^2 = 0.93$ and 0.99, respectively). It also demonstrated clinically acceptable agreement with that in A. In conclusion, arterialized SV blood samples can replace arterial blood gas samples for the measurement of pH, $PCO_2$, [$HCO_3-$], and base excess in well-perfused dogs for clinical purposes.